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Kimberley A. Jaggers,[1] *H.B.Sc. and Tracy L. Rogers,*[1] *Ph.D.*

The Effects of Soil Environment on Postmortem Interval: A Macroscopic Analysis

ABSTRACT: Burial environment, in particular soil moisture, has a significant impact on the type, rate, and extent of bone degradation, which ultimately affects estimations of the postmortem interval (PMI). The purpose of this research is to determine the effects of soil moisture on the color, weight, condition, and texture of bone as it relates to the PMI. Bone changes occurring over two different time intervals (2 and 5 months) were examined using 120 *sus scrofa* leg bones. During each time interval bones were buried in two soil environments, one of which was drier than the other. The bones in both environments lost weight over time but the net weight loss was greater for bones in the higher moisture environment. There was no change in color, texture, or overall condition, indicating that 150 days is not long enough for such alterations to occur, regardless of the moisture level of the burial environment.

KEYWORDS: forensic science, forensic anthropology, postmortem interval, skeletonization, decomposition, burial, soil moisture

To date, most of the research on estimating postmortem interval (PMI) has focused on the stages of tissue decomposition and the influence that environmental factors, such as temperature and carrion feeders, have on this process (1). There is very little information in the literature regarding the determination of the PMI once the human remains have become skeletonized (2). Most of the literature that does exist focuses on changes observed in bone biochemistry and skeletal microstructure (3–5). Although microscopic analysis can provide some useful PMI information, the processes involved with these methods have several drawbacks that necessitate alternative methods for determining time since death. For instance, many require sophisticated and expensive equipment that may not be practical for smaller police forces with limited budgets and restricted access to forensic anthropologists. In addition, many of the preliminary procedures preformed to prepare the specimen for analysis, such as sectioning, are destructive (3). Destruction of skeletal materials is of particular consequence in cases with limited remains as it results in less material being available for retesting or returning to the family of the victim. Conversely, macroscopic analysis is nondestructive, repeatable, and can be performed with limited resources and training, yet few studies have made use of macroscopic criteria as a means of determining the PMI for contemporary skeletal remains (6). The limited use of macroscopic criteria may be due in part to the inherently subjective nature of such observations and the difficulty in quantifying the results.

Taphonomic factors such as carnivore scavenging and environmental damage leave clear macroscopic evidence on the skeleton. Similarly, in burial environments soil, in particular its pH and moisture level, can have an effect on the rate of decomposition and the degree of bone erosion (4,6–13). Moisture becomes a particular issue in geographical areas with drastic seasonal changes because of the effect of cryoturbation (14), a soil disturbance caused by the freeze-thaw cycle that is dependent on the water content of the soil (14). Moisture has also been found to affect the leaching of chemicals from bone, increase dissolution and the loss of bone mineral, and facilitate the exchange of ions between bone and soil (4,5). The moisture level of the soil is affected by its composition. Soils high in clay content retain moisture while those high in sand encourage drainage of ground water (9,15,16).

Although soil moisture is understood to affect decomposition, there are no publications detailing the use of morphological criteria to determine the effects of moisture content on the degradation of contemporary skeletal remains. Furthermore, the time it takes for soil moisture to have a significant effect on skeletal decomposition has yet to be quantified. To remedy this situation, the purpose of this study is to document the morphological changes that occur to buried skeletal remains over periods of 60 and 150 days and to determine if it is possible to establish PMI based on these criteria. It is hypothesized that buried remains will show morphological evidence of degradation over one, or both, of the time intervals and that the different soil moisture levels will have an affect on the extent, rate, and/or types of degradation that occurs.

Materials and Methods

The bone specimens used in this research were *Sus scrofa* (domestic pig) femur, tibia, fibula, and patella. The use of domestic pig remains is appropriate because research has shown that pig bones are effective analogs for human bones due to compositional similarities (17) and the fact that they have a body mass of greater than 5 kg (18). The animals from which the samples came were slaughtered for food 2 days prior to pick-up and had been refrigerated up until that time. The initial stage of analysis took place 3 days after the bones were received. The bones were frozen between the time of pickup and analysis to retard the growth of mold and bacteria. Preliminary research has shown that bones can be kept frozen for up to 2 weeks and thawed without factors such as color being altered. All of the skeletal elements were partially de-fleshed, with tissue still remaining around the articulation points of the four bones.

Due to the limited resources of this particular study, the decision was made to use a large number of individual elements instead of a small number of complete pig cadavers. Had the choice been made to use a small number of complete pigs, each bone would

[1]Department of Anthropology, University of Toronto at Mississauga, 3359 Mississauga Rd. N., Mississauga, ON, Canada L5L 1C6.

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still have to be analyzed individually and fewer results would be available for interpretation. Furthermore, the results would likely have shown only that different skeletal elements degrade at different rates—a fact that is already supported by current literature (19). By contrast, using a larger number of the same type of element will provide results that are far more consistent, reliable, and statistically significant (19). The femur, tibia, fibula, and patella, in particular, were chosen for this study since it was reported that lower limb bones are among the regions most often recovered during a death investigation (20).

A sample size of 120 specimens was chosen so that each subset would have a sample size of 30 specimens. Based on other experiments conducted on human and faunal remains and given most statistical requirements, 30 is an adequate sample size (8,16,21,22). A sample size greater than 30 per burial condition would not be feasible given the limited resources and time constraints inherent in this study; and a smaller sample size may not yield results that are statistically significant.

The 120 specimens were randomly divided into two groups of 60 which were designated as group A or B and assigned to two different soil environments. Group A was buried in soil with a higher moisture content (site A) and group B were buried in soil with a lower moisture content (site B). The specimens were identified by a unique identifier, consisting of the group letter and a number from 1 to 60. The specimen number was written on a grave marker (a wooden post driven into the ground at the head of the grave) and on the bone itself using a laminated tag tied around the diaphysis of the femur. The two sites (A and B) used for this study were both located within the research forest on the University of Toronto at Mississauga campus, Mississauga, ON. The locations of sites A and B were chosen for two main reasons. First, the two sites are close enough to each other that they experience the same weather conditions and both are in partial shade, which further controls for temperature variation. Second, both sites exhibit many of the typical dumpsite characteristics including: near a road, accessible by flashlight, secluded, and use of preexisting features (in the case of site A) (13).

The soil composition at sites A and B was tested during the fall of 2006 and was found to have the following compositions: site A = 50.8% sand, 34.4% silt, and 14.8% clay; site B = 78.4% sand, 15.6% silt, and 6% clay (6). The soil at site A was determined to be loam and has a slower drainage rate (and thus, higher moisture content) due to the higher percentage of clay. The soil at site B was determined to be loamy sand and has a faster drainage rate due to the higher percent of sand and lower percent of clay. Both sites were also found to have a neutral pH of 7 (6). Studies have shown that soil pH can affect the extent and rate of decomposition (4,7–13,18). By insuring that both sites A and B have a neutral pH, the probability of introducing the confounding variable of soil acidity into the results is greatly reduced.

Each site consisted of 30 graves that were dug to be *c*. 30 cm long, 20 cm wide, and 30 cm deep and spaced *c*. 30 cm apart. Investigation into the characteristics of clandestine graves showed that a typical burial ranges from 1–3 feet in depth (12,23). In addition, at a depth of *c*. 1 foot the temperature of the soil can be expected to be close to that of the ambient temperature (9). This allowed the temperature of the graves to be estimated without disturbing the burial environment. All the graves were covered with a length of chicken wire to deter scavenging of the bones. The chicken wire extended at least 30 cm beyond the edge of the graves in all directions and was held in place with fence ties. Two specimens were placed in the anatomically correct position (i.e., side by side, both femora facing the same direction) in each grave. The use of two specimens per grave reduces the potential for bias. If both specimens within a grave show similar morphological changes it would be reasonable to conclude that these changes are the result of specimen's environment and not due to chance or the bone itself. If only one specimen shows these changes, however, it may suggest a confounding variable and would encourage a closer look at the data.

The use of separate graves for each specimen pair (as opposed to one grave for all the bones) was chosen to reduce the impact of a single grave having a unique soil environment. The use of multiple graves makes it easier for the researcher to identify if some bones show changes that are inconsistent with others. The difference in morphological condition may signify that the soil in that localized area has unusual properties that produce an exception to the more "typical" erosion patterns. Using one grave would not reveal this type of information and may introduce a bias into the results. In addition, mass graves may have effects on bone beyond that of just soil type and time, which would make it impossible to generalize the results to the single graves most typically found during a death investigation.

After interval 1 (60 days of burial), half the graves from site A and half the graves from site B were excavated. Due to animal scavenging, only 23 sets of leg bones were recovered from site A (all 30 were recovered from site B). After interval 2 (150 days of burial), the remaining graves from sites A and B were excavated. Due to animal scavenging, only 14 sets of leg bones were recovered from site A and 26 sets from site B. These four groups of 23, 30, 14, and 26 skeletal elements (comprising 372 skeletal elements) constituted the subsamples used for comparing the changes observed on the specimens between the two time periods, as well as between the two soil environments.

The macroscopic changes recorded for each skeletal element were grouped based on the following morphological categories: color, texture, amount of soft tissue, condition, number of cracks, length of cracks, weight, and hydration. The categories were defined by the following characteristics: the most consistent colors observed on the bones were expressed by their best match to the Munsell Color Chart (24) and designated using the numerical and letter code of the hue, value, and chroma; texture described the surface feel of the bone such as smooth, grainy, pitted, or porous; amount of soft tissue was recorded by location and as an estimated percent; condition referred to the level of fragmentation of the bone such as splitting, cracking, or flaking; number of cracks was determined by a numerical count of longitudinal cracks observed for each specimen; length of cracks was determined using a ruler and expressed in millimeters; weight was a measure of the dry bone in grams; hydration was a measure of the difference between wet weight and dry weight measured in grams.

All observations were recorded from dry bone, with the exception of the wet weight needed to calculate hydration. The decision to allow the bones to dry prior to making observations is based on the idea that forensic remains are more likely to be dry during the examination by an expert and thus, will more closely mimic a nonexperimental setting. To ensure that cracks did not result from drying, all bones were examined prior to drying and all observed cracks were recorded. These particular descriptive variables were chosen because they can be readily observed with the naked eye and require equipment no more sophisticated than a scale and a color chart, which allows individuals with limited resources to analyze the remains and protects the bones from destruction or damage.

The descriptive data was collected during three stages. The initial data collection took place before the specimens were buried

(October 18, 2007 for site A and October 20, 2007 for site B) and the macroscopic characteristics, as well as the weight, were recorded. After 60 days (December 15, 2007 for site A and December 17, 2007 for site B), 15 graves were excavated from each site. Once these 53 specimens were removed from the ground, they were cleaned and as much soft tissue as possible was removed (some of the tissue around the articular surfaces could not be removed without causing damage to the bone). The bones were then weighed, photographed, and placed in a fumehood until dry (18 days). The fumehood was left off to allow the bones to dry naturally and the specimens were monitored on a periodic basis to ensure that the bones did not over-dry. Once dried, the elements were reweighed, photographed, and the macroscopic observations were recorded. After 150 days (March 14, 2008 for site A and March 16, 2008 for site B), the remaining 15 graves per site were excavated. Once the 40 specimens were removed from the ground, the same procedure as used for interval 1 was followed.

Results

Interval 1 (60 Days)

Although both sites had snow covering the ground, only the soil of site B (lower moisture) showed signs of freezing. The frozen soil of site B extended *c*. 25 cm in depth, but had not reached the level of the bones in any of the graves. Daily average temperature readings for October 2007 through March 2008 were obtained from the University of Toronto at Mississauga meteorological station. The average temperature for each month of burial is displayed in Table 1.

Preliminary observation of the bones *in situ* did not reveal any obvious signs of change, nor was insect activity observed. The soil at site A (higher moisture) had not yet frozen and was soft and loose. Once the level of the bones had been reached, extensive maggot activity was observed in the majority of the graves. Initial observation suggested that the amount of soft tissue remaining on the bones differed considerably between bones of sites A and B, with site B having a much higher estimated percentage. Figure 1 illustrates the amount of tissue difference observed between the sites. A more detailed examination of all the bones from sites A and B revealed no consistent pattern of coloration within the group and no significant difference in the degree of soil staining between groups. One specimen from site A and three specimens from site B showed minimal cracking with an average size of 3.56 cm. With this exception, none of the bones showed significant texture or condition changes either within or between groups.

The quantitative variables (weight and hydration) were analyzed using a *t*-test to assess whether the mean were significantly different over the first time interval and between the two soil environments. The mean initial weight of the bones (prior to burial) was compared to that of the weight after 60 days (subsequent to the removal of the remaining flesh and drying of the bones). The results of the *t*-test showed that the bones from both sites A and B lost mass over the 60 days ($p < 0.0005$) but that the bones of site A lost significantly more mass than those from site B ($p < 0.005$). To ensure that this difference in weight loss was not the result of an original weight difference between the bones buried at each of the sites, a *t*-test was preformed. The results showed that there was no significant difference in the initial weights of the bones buried at site A compared to those buried at site B ($p > 0.5$). The *t*-tests also revealed that there was no significant difference in the hydration experienced by the bones in site A compared with those of site B ($p > 0.25$).

TABLE 1—*Average monthly temperatures for burial sites locations.*

Month	Average Temperature (°C)
October (2007)	12.89
November	2.31
December	−2.35
January (2008)	−2.30
February	−5.07
March	−1.70

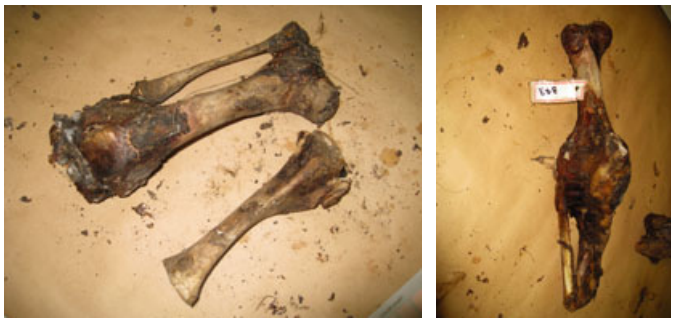

FIG. 1—*Bone excavated from site A (left) and bone excavated from site B (right) after first interval (60 days).*

Interval 2 (150 Days)

The remaining graves of both sites were excavated on March 14 and 16, 2008. Both sites had an extensive snow covering, ranging from *c*. 60 to 90 cm. The graves of site B had *c*. 30 cm of frozen soil and the freezing had penetrated to the level of the bones in one grave. Preliminary observation of the bones *in situ* did not reveal any obvious signs of change, nor was any insect activity observed. The graves of site A showed *c*. 15 cm of frozen soil but the soil directly surrounding the bone was moist and had a clay-like consistency. No active insects were observed but several dead maggots as well as pupa casings were found within many of the graves. The most notable difference observed when comparing the two sites was that the bones from site A were almost devoid of flesh. As a result of the loss of soft tissue, the femur, tibia, fibula, and patella had become disarticulated, as had the unfused epiphyses of the femora and tibia (Fig. 2). A more detailed examination of all the bones from sites A and B revealed no consistent pattern of coloration within the groups and no significant difference in the degree of soil staining between groups. None of the bones showed

FIG. 2—*Bone excavated from site A (left) and bone excavated from site B (right) after second interval (150 days).*

significant texture or condition changes either within or between groups.

The qualitative variables (weight and hydration) were analyzed using a *t*-test to assess whether the mean were significantly different over the second time interval and between the two soil environments. The results of the *t*-tests showed that bones of both sites A and B continued to lose mass over the second interval ($p < 0.0005$) and that significantly more weight was lost for both groups by 150 days compared to 60 days ($p < 0.0005$). As with the first interval, the bones of site A lost significantly more mass than bones from site B ($0.01 > p > 0.005$) (Fig. 3). There was also found to be no statistically significant difference between the hydration experienced by the bones at site A compared to those at site B after 150 days ($p > 0.05$).

Discussion

One of the most notable differences between the sites was the extensive insect activity seen at site A. This could be due in part to the fact that on October 24, 2007, 25 of the 30 graves at site A had been scavenged. Many of the bones from these graves had been scattered around the site and in the surrounding forested areas. Of the 60 sets of leg bones originally buried at the site, 23 were not recovered. The 37 sets of bones that were located were left on the ground and covered with chicken wire until they could be reburied the following morning. This exposure would have allowed for oviposit by carrion flies, which would have contributed significantly to the maggot activity found after 60 days. Out of the five graves that had not been disturbed, only one showed evidence of insect activity after 60 days. This suggests that the scavenging significantly increased the bones, exposure to oviposit by insects.

To evaluate the effect of scavenging and the resulting insect activity as a confounding variable, a comparison was made within site A between the bones' in undisturbed graves and those that had been scavenged and reburied. The most substantial impact of the insect activity would have been the loss of soft tissue. As such, the amount of soft tissue remaining on the bones of unscavenged graves was compared to bones of scavenged graves after 60 days and 150 days (Fig. 4). Figure 4 illustrates that the amount of soft tissue remaining after 60 days did not differ significantly between scavenged and unscavenged graves. This would suggest that, although insect activity would likely have been a contributing factor, it did not have a significant impact on the tissue loss within site A. Following from these observations, it can be reasoned that any differences in the amount of soft tissue remaining on bones of sites A compared to site B could be attributed to the differences in soil moisture.

To further ensure that comparisons could be made between sites A and B without introducing bias, a *t*-test was preformed to assess the difference in weight loss between scavenged and nonscavenged bones of site A. The results of the *t*-test showed that there is no significant difference between the net weight loss experienced by bones that had been scavenged and those that had not been scavenged ($p > 0.50$). This result suggests that the scavenging and the insect activity had little effect on the weight change seen within site A, supporting the position that any differences observed between sites A and B can be attributed to the differences in soil moisture.

The results of the *t*-tests illustrated three key points: (i) buried bone will lose mass over time, regardless of the soil moisture of its environment. (ii) bones in higher moisture environments will lose significantly more weight than those in a low moisture environment over 150 days. The greater loss of mass in a high moisture environment might be because of a loss of bone mineral or from leaching of chemicals from bone and the exchange of ions between bone and soil (4,5). (iii) The results showed that bones in a high moisture environment do not absorb significantly more water than those in a low moisture environment over 150 days.

Macroscopic bone characteristics of color, texture, and condition did not change over 150 days, regardless of the soil environment they were buried in. From this, two main conclusions can be drawn. First, at an average temperature of just above freezing, leg bones will not show a difference in color, texture, or condition after 150 days post-skeletonization. The fact that no morphological changes are seen on bone after 5 months raises the question of how much time is actually necessary for changes to occur at these temperatures. A more comprehensive study with a broader time span is needed to investigate temporal influences on bone decomposition. The second conclusion that can be drawn from the qualitative analysis is that the moisture content of soil has no significant effect on the color, texture, or condition of bone over a period of 150 days. The majority of the literature that discusses the impact of soil moisture suggests that it is one of the most significant factors on the rate of skeletal decomposition (4,7–13). What is not consistent in the literature is *how* moisture affects the rate of decomposition. Several studies (12,13) suggest that high moisture conditions can retard the rate of decomposition and that soils high in clay can promote preservation. Additional studies (4,5,8,11) however, suggest that skeletal remains subject to wet burial environments can degrade rapidly and be destroyed within a few years (11). The results of this study have shown that within the first 5 months after skeletonization, moisture level of soil only impacts weight; morphological characteristics such as color, texture, or condition are

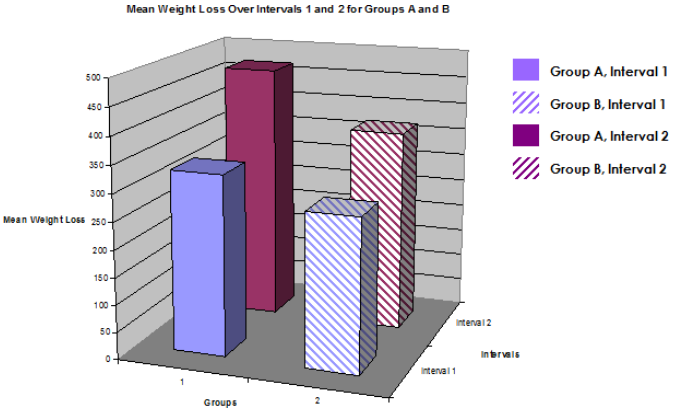

FIG. 3—*Mean weight loss over intervals 1 and 2 for bones from sites A and B.*

FIG. 4—*Bone from unscavenged grave excavated from site A (left) and bone from scavenged grave excavated from site A (right).*

unaffected. The results of this study and the conflicting observations within the current literature encourages a closer look at how moisture affects skeletal decomposition in different environments and the time it takes for moisture to impact this process.

As would be expected (1,2,11,18,21), the amount of soft tissue remaining on the bones decreased between the first and the second interval. What is of particular interest is that the bones from site A lost considerably more tissue over the two intervals than those from site B. This result supports the contention that a high moisture environment increases the rate of soft tissue decomposition (1,11,22). Interestingly, adipocere did not form on any of the remains. This could have been due to the minimal amount of adipose tissue remaining on the bones (the soft tissue that remained prior to burial was primarily ligament and tendon) (23). The average temperature at which the bones decomposed may have also factored into the lack of development of adipocere. Research shows that cold burial environments (those at 4°C or under) are not conducive to adipocere formation (23).

Two important factors must be considered when interpreting the results from this study: the influence of temperature and the fact that the results contribute to the *post-skeletonization* component of the PMI. Many studies have demonstrated that soil temperature can have a profound effect on the rate of soft tissue decomposition (2,11–13,21,25). For this reason, knowing the weather conditions for the time period of interest is necessary if any meaningful conclusions are to be drawn in regard to the PMI. The complex relationship that exists between decomposition and temperature also illustrates the importance of being cautious when applying experimental results obtained in one region to different geographical areas. The application of the results from this study would therefore be restricted to areas of colder climate and to times of the year that experience temperatures around the freezing point. In order to fully understand the impact of temperature on bone decomposition, studies such as this (also see: 8,11,16,20,21,26) should be performed in a variety of climates and seasons. Specifically, a follow-up study to the one presented here should be conducted to investigate the applicability of these results in the spring and summer months.

When using the morphological changes for skeletal remains documented in this study, it must be remembered that this describes only a small part of the decomposition process. The conclusions drawn from the data gathered in this study are only meaningful when they are combined with an estimate of the time it takes for the bones to become skeletonized. Additional data would need to be found on the amount of time required for the buried remains to become skeletonized under the given environmental conditions. For example, a study conducted by Vass et al. (26) concluded that it takes 1285 ± 110 accumulated degree days for a corpse to become skeletonized on the surface. Since the time since death estimations produced from Vass et al.'s study are based on surface remains, the result must be adjusted to reflect that of buried remains. Rodriguez (11) suggests that buried remains decompose at a rate of eight times slower than that of surface remains in the same environment. Specifically, Rodriguez estimates that in most North American latitudes, complete skeletonization of a body buried at a shallow depth of *c.* 1 foot, takes 6 months to a year or more (11). The current research suggests it will take at least an additional 5 months for significant morphological changes to begin appearing on bone.

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Additional information—reprints not available from author:
Kimberley A. Jaggers
267 Coleridge Ave
Toronto ON M4C 4J2
E-mail: kim.jaggers@utoronto.ca