Field Notes: A Journal of Collegiate Anthropology

Volume 9 Article 4

2017

Effects of Varying Levels of Soil pH on the Preservation and Appearance of Gallus gallus domesticus

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Abstract: Results from past studies noted that the carnivore digestion process results in the enlargement of foramina and expansion of Haversian canals within bones. However, it is not known whether acid erosion from soil produces similar signatures. Although bones are oftentimes found within soil matrices, some at highly acidic levels, and the time spent therein undoubtedly affects the preservation and appearance of the remains, these taphonomic effects are still poorly understood. Most studies on bone surface modification focused on how soil affects bone, particularly the diagenic processes involved, such as root and insect activity. The processes studied included root activity, insect movement, and geologic processes, while mostly ignoring the effects of the actual soil itself. Studies of soil erosion on bones have mainly focused on gastric erosion from carnivore ingestion, but the effects of acidity from soil pH levels is still a poorly-studied area within archaeology. The present study aimed to help elucidate this process by examining the effects of erosion due to soil acidity in a controlled environment. *Gallus gallus domestics* bones (n=24) were placed in containers filled with soil, whose pH values ranged from 4.5-12.6 for five weeks. This study has far-reaching implications within the field of anthropology, expanding upon previous comparative work on taphonomic signatures from hominid-modified and gastrically-modified remains recovered from fossil assemblages. The results of this study added to the literature base on the ways in which soil acidity affects the appearance of skeletal remains. Results from this study indicated that soil acidity expanded the grooves for *biceps brachii* muscle attachment like previously observed expansion of foramina from effects of gastric erosion, thus highlighting the need for further research into this process.

Keywords: Taphonomy, Soil Erosion, Avian Taphonomy

Introduction

The present study aimed to better understand the ways in which the varying levels of pH found within soil affect the preservation of skeletal remains and the degree to which soil acidity may alter bone surface characteristics during the preservation process; this question has broad implications for the fields of archaeology and taphonomy. This project also explored the ways in which soil can alter bone, including producing marks which can mimic those left by early humans and past hominins (D'Errico and Villa 1997, 28).

Bones, because of intentional burial, simple disposal, or mere chance, are often found within soil matrices, but in many archaeological studies, the effects of the soil itself are often neglected in favor of secondary influences on burials such as bioturbation or animal disturbance. Despite the enormity of skeletal remains preserved within soil, and the implications for the possible effects the acidity of the soil could have on remains, the effects of this naturally-occurring agent are very poorly studied, and even more poorly understood. Most studies concerning the ways in which skeletal remains and soil interact have dealt solely with the secondary processes concerned, such as disturbance from nearby root growth, insect movement, and geological processes which occur around the placement of the bone (Armour-Chelu and Andrews 1991, 433; Steele et al. 2015, 172). This paper aimed to address this gap within the literature.

First, this paper explored the ways in which soil acidity causes pitting on the surfaces of bones, and if this pitting resembles the pitting found in previous studies of gastric erosion. The author documented the size of the pitting present on all humeral bones before and after exposure to acid using a Dino-Lite microscope to document and take measurements of the biceps brachii muscle attachment site. D'errico and Villa (1997) provided measurements for the size of expanded foramina because of gastric erosion, and using their approach, the size of the pits seen on the bones in this experiment were compared with their data to check for correlations in expansion size (D'errico and Villa 1997, 5).

Second, this paper addressed the general ways in which five weeks within acidic and alkaline soil affected the overall appearance of the bones. The amount of abrasion marks seen on the surfaces of the bone, the amount of cortical erosion seen on the bones, and the degree of external staining seen on the bones served as metrics to measure the overall change observed during the five-week period. For the abrasion marks, the author compared the bones against other bones known to have been exposed to water, since the bones used in this experiment were within waterlogged soil for five weeks. To quantify cortical damage, a categorical ranking system was utilized, following the methods set by Gordon and Buikstra (1983, 568). The null hypothesis is that noticeable damage to the surface of the cortical bone will easily be observed, even in the short time frame of this study (Higgins 1999, 1450). The Munsell Soil Color Chart, whose use has a long history within the discipline of forensic anthropology served to assess the degree of staining on the external surfaces of the bones (Shipman et al. 1984, 309).

Finally, this paper examined the broader implications of these markings and highlighted the need for further research into the ways soil acidity affects bone decomposition. Previous research demonstrated that acid from carnivore's stomachs can produce pitting on bones, but the degree to which the pitting from stomach acid resembles pitting from soil's acidity remains uncertain (Fernandez-Jalvo et al. 2014, 323). Findings from D'errico and Villa (1997, 27) demonstrated the need to re-examine commonly held ideas of hominin markings due to new data on gastric erosion.

Their paper served as the basis for this study and the proposal for the need to further research into whether acidic soil erosion can produce the same type of pitting on bones previously misconstrued as hominin modification.

Background

A seminal study on the ways in which the bones preserved within soil are affected by the surrounding pH levels was conducted by Gordon and Buikstra in 1983. Their study aimed to determine how much weathering and destruction of bone occurred after burial because of acid erosion from the soil's pH levels, and how much the said pH affects estimates of time since initial burial (Gordon and Buikstra 1983, 566). They utilized a categorical scoring system for their analysis, ranging from one for bones that were "undamaged", to five, for bones so degraded as to be unrecognizable (Gordon and Buikstra 1983, 568). Unfortunately, inquiries into soil acidity and its effects on bones seems to have been mostly confined to this early work. Most studies since then focused more on extraneous factors associated with burial, such as bioturbation, rodent interference, or diagenesis (Berna et al. 2004, 877; Nicholson 1996:514; Trueman et al. 2004, 728). Much of the literature concerned with studying soils at archaeological sites deals with the chemical traces found within the soil; one of the most common elements studied being phosphorous, as it is a sensitive and persistent indicator of human activity (Cavanagh et al.1988, 67; Holliday and Gartner 2006, 301; Parnell et al. 2002, 380; Wilson et al. 2008, 421). However, as the Gordon and Buikstra work demonstrated, acidity from soil does play a major role in the preservation and appearance of bones found at archaeological sites, and its exact influence deserves broader study within the field of taphonomy.

In a study performed by D'errico and Villa (1997, 9), the authors reexamined previously held notions of early instances of bone art made by Upper and Middle Paleolithic groups. Their study found that holes present on bones previously thought to be carved as decorative features in fact show many similarities to holes made from gastric erosion after carnivore ingestion (D'errico and Villa 1997, 14). They analyzed sites across Europe with faunal remains known for their anthropic modification, comparing them against bones chemically modified in the lab under conditions meant to mimic gastric erosion. The results demonstrated that lab-induced acid erosion on bone surfaces is like what is seen under natural conditions, and further, that many of the larger holes from samples appeared to be the result of expansion in nutrient foramina. However, the stomach acid of generalist carnivores is quite acidic, being around 2.2 pH, and so the holes seen in their study were most likely more extreme than what will be witnessed in the present one (Beasley et al. 2015, 4).

Effects of gastric erosion were further examined in a study by Fernandez-Jalvo et al. (2014, 331), looking at the differences between abrasion marks and digestion marks, which are "two taphonomic agents [that] can be difficult to distinguish" (Fernandez-Jalvo et al. 2014, 323). Their study utilized a combination of hydrochloric acid and enzymes to mimic the natural digestion process, sometimes using both agents and sometimes using only one at a time. Results from Fernandez-Jalvo et al. (2014, 331) show the combination of hydrochloric acid and enzymes can yield results like what is seen in bones after mammalian or avian ingestion, though at a slower rate and less pronounced than in natural circumstances. The current study aims to follow in the footsteps of this earlier study and continue exploring this little-understood topic.

Though ideal for this experiment due to ease of accessibility, the use of avian bones comes with a few caveats, namely , that the biomechanical properties of avian bones differ from many other types of bones. Furthermore, per Higgins (1999, 1449), avian remains are largely overlooked in many taphonomic analyses, possibly due to the low energy gains to be had from breaking down and consuming such small animals, and possibly because of the difficulty in accurately identifying such small remains. However, avian remains are helpful in assessing past behaviors, and their presence at sites is a subject which warrants greater review, starting with the current study. The following section provides a brief overview of avian osteology to aid in the understanding of how acid erosion affects avian bones specifically.

The bones used within this experiment are comprised of *Gallus gallus domestics* humeri, radii, and ulnae. The humeri of avians is particularly interesting, as it is what is referred to as a pneumatized bone, meaning the bone is comprised of "a diverticulum, or air sac, within the marrow cavity" (Higgins 1999, 1452). This pneumatization of the long bones in avians mean the outer cortical wall of bones is also thinner than non-pneumatized bones, likely leading to faster erosion rates of the cortical wall than seen in other animals. However, as noted later, further experiments are needed to test if avian bones do indeed erode faster than those of animals with thicker cortical walls.

Methods and materials

To examine the ways in which soil acidity affects bone preservation, the author placed 24 bones from *Gallus gallus domesticus* humeri, ulnae, and radii into six containers containing top soil and chemically altered the pH values of five of these containers, leaving the pH value of one container at its starting value of 8.4**.** The bones used were a mixture of humeri (n=15), ulnae (n=2), and radii (n=7). Bones for this experiment were obtained from several sources. The bones used for this project came from Buffalo Wild Wings, who generously offered to donate 20 chicken wings, consisting of ulnae, humeri, and radii, . All bones used for this study exhibited fully-fused epiphyseal ends, suggesting the specimens were adults at the time of death (Deng et al. 2014, 190). Nicholson (1995, 513) noted that the bones of juvenile specimens decay faster than their adult counterparts, which prompted the use of adult specimens for this study. Bones were boiled for approximately five hours at the low setting (approximately 190 ° F) in a crock pot. Roberts et al. (2002, 492) noted that boiling bones for less than nine hours produced little effect on the chemical composition of the bone, so the cooking time should not influence the results seen in this study. Excess tissue was removed by hand, and the bones were wiped down with a wash cloth afterwards. A sample size of 24 ensured the bones could easily be divided within six different containers, with four bones in each container.

A bag of topsoil was used in this study. Originally, the author intended to gather local soil from around the area, but discarded this idea due to probable inconsistencies with the starting pH levels of different soil samples; further, buying topsoil from a store ensured knowledge of the exact ingredients within the soil going into the project. The author divided the soil into different plastic containers, placing one cup of soil within each container. One cup of distilled water was then added to the cups of soil placed within the plastic containers. This 1:1 ratio for testing pH is an established method for testing pH levels. Distilled water was chosen over tap or bottled water because it has a more neutral pH than these alternatives (Osman 2013, 106). Water was obtained from a local grocery store. The pH meter came from Amazon and was manufactured by the company, Luckystone. The pH meter was calibrated per instructions provided by the manufacturing company. The manufacturing company recommends this be done roughly every month, so the meter did not need to be calibrated a second time, as the bones were only placed within the soil for a five-week period.

After calibrating the pH meter, the author then proceeded to attain the proper pH levels for soil samples. Four different products were obtained to alter pH levels. The products pH up and pH down are both intended for hydroponic gardening use, sold by the company General Hydroponics. In addition to these products, hydrochloric acid (HCL) and sodium hydroxide ($NaOH^+$) were obtained from a local chemical supply store. These two chemicals are the most extreme that the author could acquire for this project, with HCL having an acidity of 1, and $NaOH^+$ having an alkaline pH of 14. Initial research designs included pH ranges from 1-14, with one container at a constant pH level of 7, but after several days of unsuccessful attempts to achieve these levels, the decision was made to refine the original idea to avoid adding too many chemicals to such a small amount of soil, which could potentially cause unintended effects on the bones from such heavy levels of chemicals. The final levels of pH for each container were 4.5, 5.9, 6.8, 8.4 (the starting pH of the soil), 9.3, and 12.6. The values fluctuated throughout the week, and so the levels were checked weekly and adjusted accordingly. To ensure there was no cross-contamination between testing containers that might influence the reading from the pH meter, the pH meter was rinsed clean with distilled water before and after each measurement was taken.

At the onset of the experiment, the author was not aware of potential expansion of the biceps brachii muscle attachment site, and so did not have a control group for the humeri. As a result, a larger sample of humeri was obtained after removing the bones from the soil containers at the end of the five-week period. The author measured the average size of the attachment site before exposure to pH with the Dino-Lite measuring tool. Starting values for muscle attachment site were then compared against the end results from the bones placed within soil.

Measurements of the size of the muscle attachment site were taken using a Dino-Lite Microscope provided by the University of Colorado, Denver. The author compared the final size of the muscle attachment site against the starting values, and against the reported range of foramina expansion caused by gastric erosion in D'errico and Villa (1997, 23). To assess the degree of staining seen on the bones, they were scored against the Munsell Soil Color Chart per methods outlined by Shipman et al. (1984, 309). For the purposes of assessing the extent of erosion on each bone, bones were scored as belonging to any of five different categories, whose values were not discrete, meaning one bone could fall into multiple categories. Category 1 includes bones whose appearance showed no change in outward appearance throughout the project. Category 2 includes bones whose only change was a change in coloring over time, meaning there was not pitting or erosion of cortical bone. Category 3 includes bones with only minor cortical wear on one of the epiphyseal ends and expanded biceps brachii muscle attachment sites, hereafter referred to as "pitting". Category 4 includes bones with cortical damage on both ends and one pit. Category 5 includes bones whose pitting is severe enough as to show into the internal structure of the bone. These categories are based on similar ones used by Gordon and Buikstra in their analysis of erosion of bones from Late Woodland burials in Illinois (1983, 569).

Results

The bones showed signs of damage ranging from a marked discoloration of the external bone, to cortical erosion on the epiphyseal ends, and in the case of the humeri, expansion of the biceps brachii muscle attachment site. Figure 1 shows the range of variation in the erosion of bones based on these categories. Figure 2 shows evidence of cortical erosion on an epiphyseal end.

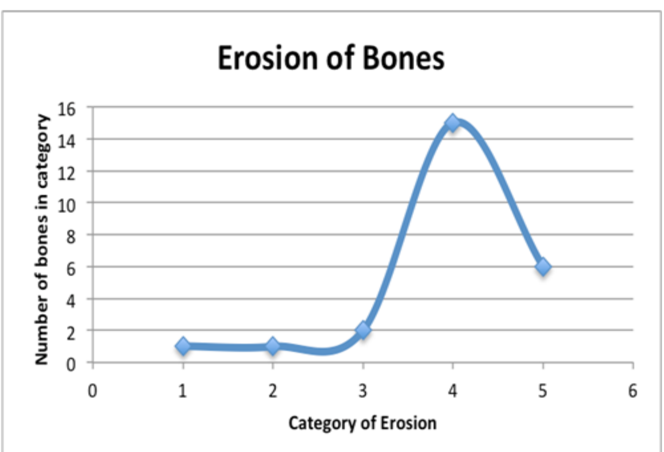

Figure 1: Distribution of erosion based on category.

Figure 2: Cortical erosion.

Results varied across the sample. The expansion of the muscle attachment site varied across humeral bones and across differing levels of pH. Some of the bones showed very little expansion, while in other bones, the expansion was quite marked. The average size of the attachment site before exposure to pH was 4.81 mm (measured by author using a Dino-Lite microscope). The average size of expansion across the sample is 7.21 mm. The range of values for the sample was 6.06 mm. No pitting was observed on bones other than the humeri, possibly because of smaller muscle attachment sites on the other bones of the forearm.

The pitting is most likely an effect from the soil, and not the added water, since the bones placed within the 8.4 pH container also showed expansion of the biceps brachii attachment site. Except for one outlier, all bones showed substantial expansion of the biceps brachii muscle attachment site. The one bone whose value, 4.74 mm, lies below the average of bones not exposed to soil, was from the most acidic sample. The attachment site on the other humerus placed within this container expanded by approximately 4.55mm. Both bones were placed within equal levels of soil for the same length of time, so the reason behind this discrepancy in results warrants further research. It is possible that this side of the bone simply was not exposed as fully to the acidic soil as the other humerus within this container. However, the bones were all placed within the same amount of soil, and care was taken to ensure all bones were covered completely at all times, so this reason is not likely. Further research would help to elucidate the reason for this anomaly.

The expansion of the muscle attachment site in the humeri of this sample was quite close to the expansion reported by D'errico and Villa in their analysis of gastric erosion on bones (D'errico and Villa 1997, 23). As noted in Figure 3, there was a rather large range in variation in overall size of the expanded attachment site, with some pitting more than double the size of that seen in other bones.

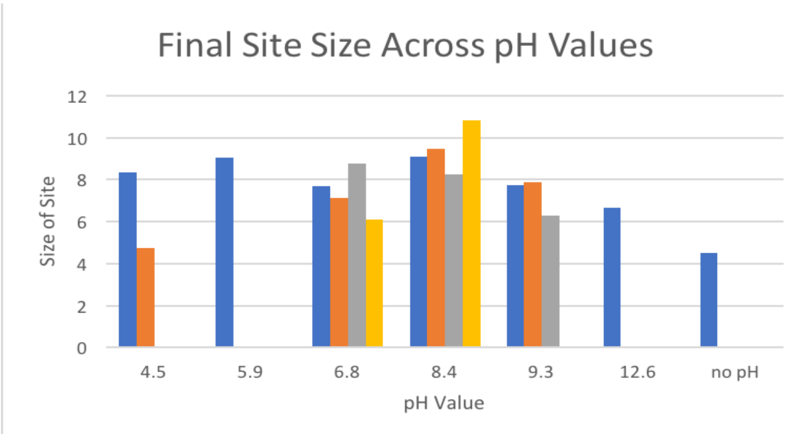

Figure 3: Range of final sizes for biceps brachii muscle site in millimeters.

While the size of the sample in which foramen expansion was noted was not specified by D'errico and Villa (1997, 23), several bones within their study did have recognizable expansion at known sites of nutrient foramina. The authors mentioned the larger holes in their sample size, up to 9.5 mm, were likely the result of foramina expansion, and this number fits with the range seen in this sample in all but one specimen. The authors made note of some bones being heavily damaged, which hampered their ability to accurately identify the exact location of the pit on the bone and further correlate this location with a pre-existing osteological feature. Figure 4 shows the expansion in one such site.

The degree of expansion for muscle attachment sites was also looked at as a percentage of the whole humerus. On average, the size of the muscle attachment site at the end of the fiveweek experiment comprised 3.30% of the total bone size, compared to 2.01% of the bones for those not exposed to any level of pH. This is a 64% increase in size on average. Both the size of the expanded attachment site and the size of the humerus were measured in millimeters.

Figure 4: Expansion at the biceps brachii attachment site from pH level 4.5.

Figure 5 shows the values for the percentage of each biceps brachii muscle attachment site compared to the total length of the bones. As for the radii and ulnae, observed changes in the external appearance of these bones included a darkening in color of the external bones, as well as slight erosion on the distal ends. All bones within this experiment, except for those within the soil container of 8.4 pH, showed signs of abrasion, as well.

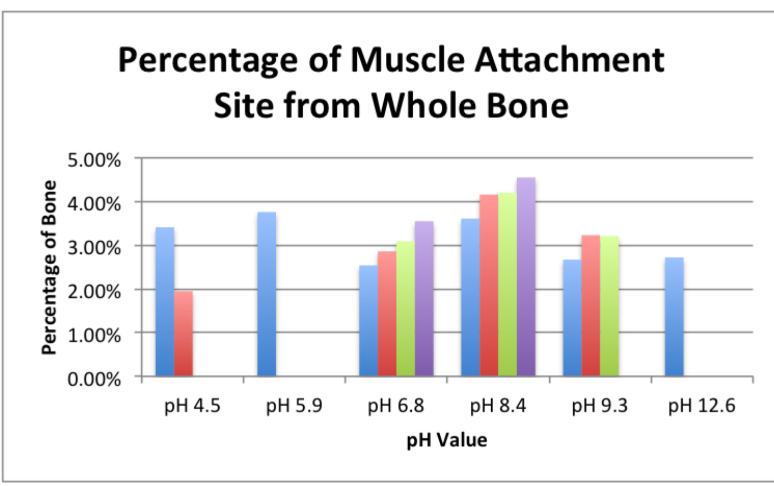

Figure 5: Percentage of expansion site versus total bone.

The lack of abrasion marks on the bones from this container is most likely attributable to the lack of water added to this soil sample. All bones showed signs of darkening color over the five weeks. The exception to this rule is the bones placed within the container that did not have added water; the bones within the 8.4 pH container retained their original color for the most part.

The final appearance of the external surface of the bone was scored as values along the Munsell Soil Color Chart, as outlined by Shipman et al. (1984, 309). Figure 6 illustrates the range of values along the Munsell Soil Color Chart. The starting code of the bones, not plotted on the chart to avoid data bias, is 2.5Y7/4. Figure 7 shows differences in final color from bones within 8.4 level soil pH, 5.9 level soil pH, and 9.3 level soil pH, respectively.

Discussion

The most notable result to come out of this study was the expansion of the biceps brachii muscle attachment site. As the average level of stomach acid pH is far below any level seen in this project, lower pH levels for the soil matrices might yield more extreme pitting results comparable to those seen in the D'errico and Villa (1997, 23) paper. Further studies looking at the effects of soil erosion on bones would determine if the expansion of the muscle attachment sites seen in this study would continue across further research projects.

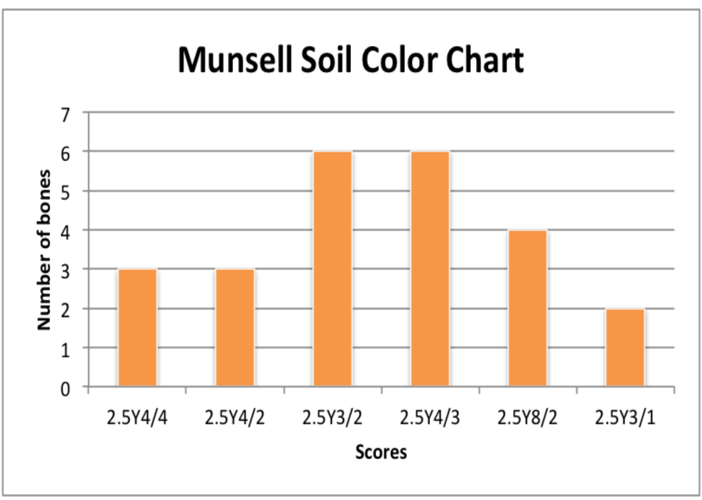

Figure 6: Distribution of Categories based on the Munsell Soil Color Chart.

However, 14 out of 15 humeri showed significant expansion, so it is likely this result is not abnormal. If these results hold true in further studies, there would be grounds to re-examine pitting found on bones as seen in the study by D'errico and Villa (1997, 23), but in the context of acidic soil erosion being the possible cause of pitting.

Figure 7: Differences in final coloring of bones from pH levels 8.4, 5.9, and 9.3, respectively.

The bones also showed slight cortical erosion on the distal and proximal ends. Erosion was scored on a presence or absence basis for the purposes of this experiment, as outlined by the Gordon and Buikstra (1983, 568) study. Further studies using SEM methods would be useful to determine the exact surface area and amount of eroded bone relative to total bone, but this technology was not available to the author at the time of this study. The use of the Munsell Soil Color Chart to assess surface staining does have its drawbacks. The method requires holding bones up to the chart to compare color values, which has the potential to lead to researcher bias. However, the method has a long history within the discipline of anthropology , which demonstrates its validity(Shipman et al. 1984, 309). Given further time and resources, a less controversial method could be employed in future studies.

Conclusion

For this study, *Gallus gallus domesticus* bones were used for expediency, but they do not have much relevance within the archaeological literature (Higgins 1999, 1449). Avian bones have thinner layers of cortical bones than many other mammals, and their rate of erosion on the cortical surface is possibly greater than what would have been observed in a five-week study using mammalian bones (Higgins 1999, 1450). However, Nicholson (1996, 521) observed that when placed in acidic soil with the bones of other animals such as rats, cows, and fish, the bones of pigeons appeared to decay at equal rates as those of non-avian species. Future research is needed to explore the possible differences in the rate of decay for bones with thicker and thinner layers of cortical bone, as well as to determine if bones from juvenile individuals erode faster than the adult specimens used in this study. The short time span of this project limited the amount of data which could be obtained from this study. Future projects would need to be prepared for possible expansion of certain features such as the biceps brachii muscle attachment site, as noted within this study, and use control groups accordingly. Additional time within acidic soil would likely yield greater rates of expansion of the muscle attachment sites, as well as possibly other features such as the expansion of the foramina, as seen in D'errico and Villa (1997, 23).

Based on the results of the study, the null hypothesis could not be rejected, as there was a difference in the appearance of the cortical bones after the five-week period. Bones from all levels of pH showed distinctive differences from the time spent within the soil, suggesting that bones are very vulnerable to damage from a spectrum of pH levels. Further research would help to elucidate further, the ways in which soil acidity affects bone decomposition and the ways soil erosion are different from or similar to gastric erosion.

Acknowledgements

Special thanks to Dr. Jamie Hodgkins who inspired this project, and whose expertise and guidance proved invaluable. Thank you to Dr. Charles Musiba for all the feedback and advice given throughout the project.

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