The Effects of pH on the Decay of Animal Bones

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Edited by Macy Strain

Abstract: It is a well-known fact in the forensic community that the process of soft tissue decomposition lasts much longer when the cadaver is submerged in an aqueous environment. Studies of this nature with skeletal remains are less frequently produced, and therefore there is not much supported knowledge about whether human bones would face similar effects. The human skeleton potentially undergoes physical changes over various environmental conditions, but their changes in liquid conditions are poorly understood. This experiment was a study on the macroscopic effects that pH may have on the degradation of animal bones. Various small animal bones were placed in common household solutions of differing pH for the purpose of recording any physical changes. The only notable changes observed over the course of the experiment were the drastic discoloration of solutions with higher acidity, as well as the production of sediment-like residue in the saltwater solution. Further investigation into this in the context of potential body disposal could prove useful for the future of forensic science.

Keywords: skeletonization, pH, human decomposition, postmortem, degradation

A human cadaver decomposes differently within varying environmental conditions – as a rule of thumb in the forensics sphere, the human decomposition process lasts about one week in air, two weeks in water, and eight weeks in soil. Much research has been conducted into the decay process of soft tissue, contributing to quite a bit of the knowledge used to estimate important information such as the approximate postmortem interval (Cockle 2013). The same cannot be said regarding the scientific community's efforts into conducting the same research over the decay processes of hard tissue.

Forensic anthropology is a growing field that poses many benefits to criminal investigation. For example, bones can provide identification of a body through DNA extraction if the remains are found under the right conditions. For example, time since burial can be a factor affecting the quality of the DNA in hard tissue remains (Duijs 2020) However, damage to the remains or degradation over time makes DNA extraction difficult or even impossible.

The decay of DNA within the human skeleton is influenced by factors like thickness of the bone, depth at which the bones were left within soil, pH, and water saturation (Allentoft 2012). This poses an important problem as human remains are typically not found in such proper conditions.

Environmental factors such as temperature, geographic location, scavengers, and attempts of clandestine body disposal can accelerate the soft tissue decomposition process, making identification and evidence collection exponentially more difficult for investigators. The human skeleton, however, remains much longer even after the process of soft tissue decomposition ends. The ability of bones to remain intact after years, decades, and even centuries is a marvel of modern science.

The human skeletal composition is made up of a majority of phosphatic minerals, along with collagen, water, and other proteins and lipids. Because of this, it inherently possesses a high pH between 8 and 10 (Pfretzschner 2004). pH, or the "potential of hydrogen", is influential in its own right; while there is a rough consensus that a human body takes much longer to decompose in soil than in water or air, that time frame is also affected by the pH. For example, acidic soils can degrade even the toughest of bones (Dent 2003).

In addition to natural decomposition, certain conditions can be manipulated in order to conceal a body. In some instances, people have been known to attempt to dissolve human remains with certain acids. Bone can easily be eroded by a solution acidic enough, and can even be almost completely digested in some cases (Vermeji 2015).

There is little known about the effects that different pH may have as the environment that surrounds human remains (Amadasi 2015). Additionally, there is not much known of the effect of housing skeletal remains in typical, everyday solutions. These solutions have significantly more neutral pH compared to the hydrofluoric and sulfuric acid that may be used to dispose of a body, meaning these household solutions may have a less dramatic effect on the condition of the bone. Noting the macroscopic effects that solutions with more neutral pH levels may have on the degradation of animal bones could prove useful to further understanding more about the human skeleton in the context of forensic investigations.

Materials and Methods

Experimental Solutions. Five different solutions were chosen for this experiment to represent a variety of pH levels. The solutions chosen were tap water, salt water solution (*H-E-B Grocery Company, TX*), carbonated soda (*Keurig Dr Pepper, TX*), oat milk (*HP Hood LLC, MA*), and brewed coffee. Plastic cups (*Hill Country Essentials, TX*) were used to contain the solutions over the duration of the experiment.

For the purposes of this experiment, the solutions were gathered several days prior to the beginning of the experiment as some were acquired in conditions other than room temperature. 12 ounces of each solution were kept in each plastic cup in an indoor room away from sunlight exposure and maintained

at a constant temperature of 72.0 degrees Fahrenheit or approximately 22.2 degrees Celsius to ensure equilibrium temperature for all the solutions.

pH Measurement. A pH meter (*Ruolan Lab)* was used to measure the pH of each solution. The pH for the tap water was 7.1, the pH for the salt water solution was 8.2, the pH for the oat milk was 6.8, the pH for the carbonated soda was 2.9, and the pH for the cold brew coffee was 5.2. Because all the solutions were household solutions and common beverages, the pH were not of extreme acidity or alkalinity.

Pre-experimental Setup. Each of the animal bones used in this study were selected for their compact size. One plastic cup was designated for each solution and labeled correspondingly.

Experimental Procedure. Once all solutions were all under the same temperature conditions, the experiment could begin. Each small animal bone was placed in its respective solution. The experimental environment was continuously monitored and maintained the same throughout the experiment in order to mitigate any external variables. The animal bones were then examined again in 12-hour intervals, starting from 9:00 PM the first day of the experiment to 9:00 PM on the third day of the experiment. Visible observations or changes were recorded during each examination. The characteristics that were observed and recorded during the experiment included discoloration and residue. After the 48-hour duration of the experiment, the animal bones were removed from the solutions and left to dry on paper towels overnight. Any additional macroscopic observations relating to discoloration or residue were made after the bones were left to dry. Finally, the rigidity of each bone was tested and recorded by striking each bone with a glass bottle five times.

Results

Before the experimental treatment, the animal bones were intact with a light umber-gray color. The animal bone that was placed in the tap water did not show any signs of obvious change. Of all the characteristics being monitored, there was essentially no difference over the duration of the experiment. The animal bone that was submerged in the saltwater solution yielded similar results, but began to deposit a visible, sediment-like residue with small black particles after the first 12 hours, as well as a very minimal yellowing in color.

The animal bones placed in the cold brew coffee and carbonated soda showed signs of obvious dark brown discoloration. For the solutions of higher alkalinity, like the tap water and saltwater solutions, there was much less visible color change. The animal bone submerged in the oat milk also yielded a change in color, but rather than becoming darker it became lighter in saturation than the others. It also began to develop a mold-like substance on the exterior some time after the first 12 hours (figures 1-5).

Table 1. Observed color and residual changes in animal bones after 12 hours in solutions of various pH.

12-Hour Observations		
Solution	**Discoloration**	**Residue**
Tap water	None	None
Salt water solution	None	Sediment-like
Oat milk	None	None
Carbonated soda	Brown	None
Cold brew coffee	Brown	None

Table 2. Observed color and residual changes in animal bones after 24 hours in solutions of various pH.

24-Hour Observations		
Solution	**Discoloration**	**Residue**
Tap water	None	None
Salt water solution	Yellowing	Sediment-like
Oat milk	Lightening	Mold-like

Solution	Discoloration	Residue
Carbonated soda	None	None
Cold brew coffee	Dark brown	None

Table 3. Observed color and residual changes in animal bones after 36 hours in solutions of various pH.

36-Hour Observations		
Solution	**Discoloration**	**Residue**
Tap water	None	None
Salt water solution	Slightly yellow	Sediment-like
Oat milk	Lightening	Mold-like
Carbonated soda	Dark brown	None
Cold brew coffee	Dark brown	None

Table 4. Observed color and residual changes in animal bones after 48 hours in solutions of various pH.

48-Hour Observations		
Solution	**Discoloration**	**Residue**
Tap water	None	None
Salt water solution	Slightly yellow	Sediment-like
Oat milk	Lightening, gray	Mold-like
Carbonated soda	Heavy, dark brown	None
Cold brew coffee	Dark brown	None

Table 5. Observed color and residual changes in animal bones after removed from solutions of various pH and dried overnight.

Observations After Drying			
Solution	**Discoloration**	**Residue**	**Rigidity**
Tap water	Small brown speckles	None	Completely intact
Salt water solution	Slightly yellow	Sediment-like with black specks	Completely intact

Solution	Discoloration	Residue	Rigidity
Oat milk	Lightning, gray and slightly yellow	Mold-like	Completely intact
Carbonated soda	Heavy, dark brown	None	Completely intact
Cold brew coffee	Dark brown	None	Completely intact

Discussion

Human tissue, no matter soft tissue or bone, can easily be modified using substances of the right pH. It is not uncommon that a body may be disposed of through use of chemical processes. Even if complete dissolution of a cadaver is unsuccessful, when dealing with skeletal remains, certain identifying marks and injuries that may have been clear before can almost completely deteriorate with the most alkaline of solutions (Amadasi 2015). Even when occurring naturally, tissue can easily undergo drastic chemical changes – nitrogen products like ammonia will be produced during tissue hydrolysis, raising the pH of the body's tissue to extremely alkaline conditions, which encourages the growth of bacteria and accelerated putrefaction (Zhou 2011). A bone even found buried can deteriorate under the right soil pH conditions. In addition to this, older bones are much less likely to be preserved due to protein loss, which will cause them to lose their integrity (Janaway 2009). Determining the impact of less extreme pH conditions on hard tissue can help us to determine the point at which bone has lost integrity in forensic investigations.

Some precautions were taken in attempting to control the experimental environment as many variables had the ability to heavily influence data. For example, temperature was kept at a recorded constant as warmer temperatures seem to catalyze the process of decomposition more often (Petrik 2004).

The most interesting outcome from this experiment was the notable color change in the carbonated soda solution as opposed to the salt water solution. These two experimental variables had the most difference in pH, with carbonated soda being acidic at a pH of 2.9 and the salt water solution being moderately alkaline at a pH of 8.2. While the animal bone in the salt water solution produced small amounts of residue, the color change was rather insignificant. On the contrary, the animal bone immersed in the carbonated soda completely changed color to a heavy, dark brown. While these bones did not necessarily undergo visible decay in the conventional sense, it would be useful to be able to microscopically inspect these samples after the experiment to potentially show any minute evidence of decay such as digested bone or the thinning of bone structure (Vermeji 2015). There is potential for further research in this aspect.

While human and animal bones have many differences, similar logistics can be applied if

this study were to be done on human bones (Aerssens 1998). Such information could prove useful in determining the value of anthropological estimations or DNA evidence even after remains are found in solutions of high acidity or alkalinity.

Had this experiment gone on for a longer duration of time, possibly weeks, results may have been more drastic. In addition to this, ambient environment that the solutions were placed in was not a completely controlled environment which may have contributed to certain changes as well. More invasive procedures of analysis such as Fourier transform infrared spectroscopy (FT-IR), optical emission spectrometry (ICP-OES), and scanning electron microscopy (SEM) could potentially yield more in-depth results (Amadasi 2017). Microscopic evaluation of bone that has been subject to less extreme pH conditions could provide more reliable characteristics that can be used in determining the evidentiary value of a bone based on deterioration. Further investigation into this concept, with solutions of more extreme acidity and alkalinity, a longer observation time frame, and more sterile conditions could provide useful knowledge in taphonomy.

Acknowledgements

I would like to thank Dr. Adrienne Brundage for your inspiration to investigate both the microscopic and the macroscopic characteristics in this experiment. I would also like to thank Rachel McNeal for providing the animal bones necessary to perform the experiment as well as Macy Strain for contacting Rachel on my behalf. Finally, I would like to thank Gabrielle Thornton for helping construct the basis of this experiment and helping to flesh out the idea.

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