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TECHNICAL NOTE

ANTHROPOLOGY

Angi M. Christensen,[1] *Ph.D. and Sarah W. Myers,*[2] *B.A.*

Macroscopic Observations of the Effects of Varying Fresh Water pH on Bone

ABSTRACT: Little is known about the decomposition of remains in aquatic environments of varying pH, and even less is known about the specific effects of these environments on bone. Bovine bones were placed in solutions of pH 1, 4, 7, 10, and 14 and observed over a period of 1 year. All solutions eventually removed or dissolved the soft tissues from the external surface of the bone. The pH 7 and pH 10 solutions had little effect on the bone, but the other solutions affected the bone to varying degrees. Extreme pH levels were the most destructive, while more moderate pH levels had lesser but significant and interesting effects. Empirical data on postmortem aquatic changes may be extremely useful in forensic contexts for both improving time since death estimates and also for providing better information to underwater recovery experts thereby potentially increasing the quantity and quality of remains recovery.

KEYWORDS: forensic science, decomposition, pH level, aquatic environment, adipocere, time since death, recovery

Numerous studies have examined the decomposition of soft tissue and the effects of varying environments on the timing and sequence of postmortem alterations. These studies have primarily examined the effects of ambient and soil conditions on muscle and organs (see [1,2] for just a few examples). Some have specifically examined bone preservation, but limit interest to terrestrial environments and differences in soil pH (3,4). Documentation of postmortem changes in aquatic environments has been scant and consists primarily of general overviews (5), a few empirical studies (6–9), and case reports (10–16). Other reports emphasize the formation and preservation of adipocere (4,17–20), algae formation, (21), invertebrate colonization (9,22), or fluvial transport (23–26). Little research has been carried out specifically on bone preservation in various aquatic environments (27,28).

The effects of extreme pH environments on bone can be seen when remains are eaten and partially digested by animals. For example, bones can degrade in a matter of days after being ingested by a shark that can have a stomach pH as low as 2 or 3 while digesting (29). Although the average pH of bodies of water is 6–8, the natural variation in aquatic pH levels is very high. In the U.S. alone, the pH levels vary from around 4.3 in Squash Lake and other lakes in the Adirondack region to pH 10 in the Great Salt Lake in Utah (30–32). There are thus a variety of aquatic environments in which human remains may be deposited and subsequently discovered.

The location and recovery of human remains in aquatic environments is a relevant and important forensic matter, and improved empirical data could prove extremely useful. Time since death is often an issue of great importance in forensic investigations, and more knowledge about the rate of decomposition and the importance of pH and other factors may improve these estimates. A perhaps less often considered benefit of improved and increased data on aquatic postmortem change relates to the actual recovery process. Divers and other recovery personnel are often faced with difficult search conditions including widely scattered and fragmentary remains, and poor visibility. A better knowledge of expected decompositional changes could help these experts prepare for the condition of the remains they are likely to encounter thus potentially improving the quantity and quality of recovery. Prompted by an inquiry about the likely state of a body in an acidic lake, and because of the lack of empirical data subsequently located, the following study was undertaken to examine the effects of varying aquatic pH environments on bone preservation.

Materials and Methods

Bovine remains were obtained from a meat processing facility and stored in 4°C refrigeration until time of use. Although other species are typically considered better human models, these remains were readily available and were deemed suitable for the macroscopic observations of this study. The specimens retained some connective tissue and muscle but no flesh. Portions of two femora were cleaned using Tergazyme® (Alconox, Inc., White Plains, NY) to remove any adhering mold, bacteria or other compounds, or debris on the surface. The bones were then cut into *c.* 3–5 cm thick cylindrical disks using a table saw.

Solutions were prepared to represent aquatic environments of different pH levels using nitric acid and sodium hydroxide solutions purchased from Fisher Scientific (Pittsburgh, PA). (Sulfuric acid would have been preferable because it likely represents the acidic component in most natural aquatic environments, but nitric acid was selected for the study because of its availability.) Nitric acid, HNO_3, Fisher Lot# 030489, represented pH 1, and HNO_3 was added to tap water until a solution of pH 4 was created. The basic solutions utilized NaOH, Fisher Lot# 062634. A 30% NaOH

[1]Federal Bureau of Investigation, Laboratory Division, Forensic Anthropology Program, 2501 Investigation Parkway, Quantico, VA 22135.
[2]Federal Bureau of Investigation, J. Edgar Hoover Building, 935 Pennsylvania Avenue NW, Washington, DC 20535-0001.

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solution was used for the pH 14 solution, and the 30% solution was added to tap water to prepare a pH 10 solution. Tap water was used for the pH 7 solution.

The specimens were placed into glass beakers and the prepared solutions were added until the bones were completely submerged. Two specimens were placed into each acid/base solution and five specimens were placed in the pH 7 solution. The beakers were covered in Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL) to minimize evaporation and placed under a ventilated hood to minimize odor dispersion. The temperature in the room where the specimens were stored was *c*. 73°F.

The specimens were periodically removed from their solutions using stainless steel spatulas and a specially prepared wire basket tool. Early in the study, specimens were photographed daily, then tapering off to approximately twice a week, weekly, every 2 weeks, and then monthly for the last 7 months. They were blotted of excess solution and then photographed in both top and side views. The pH of the experimental solutions was monitored throughout the study and was adjusted back to the original level when necessary using the nitric acid and sodium hydroxide. After 1 year, the specimens were removed from the solutions, rinsed, photographed, and examined visually and microscopically.

Results

Figures 1–5 illustrate changes observed following given time periods for each solution (i.e., "Day 1" depicts the specimens at the end of 1 day). The specimens in the highly acidic pH 1 reacted instantly (Fig. 1). Immediately after submersion in the HNO_3 solution, the specimens began to visibly degrade. After 1 day, the marrow was dissolved from the smaller of the two bone pieces. The bones also displayed noticeable grooves and ridges. After 3 days, only two small pieces remained of the smaller bone, and the larger bone's size had been significantly reduced. Size reduction continued through the first 2 weeks. By the end of the second week, both bones were almost completely dissolved with only a few remnants remaining floating on the top of the solution.

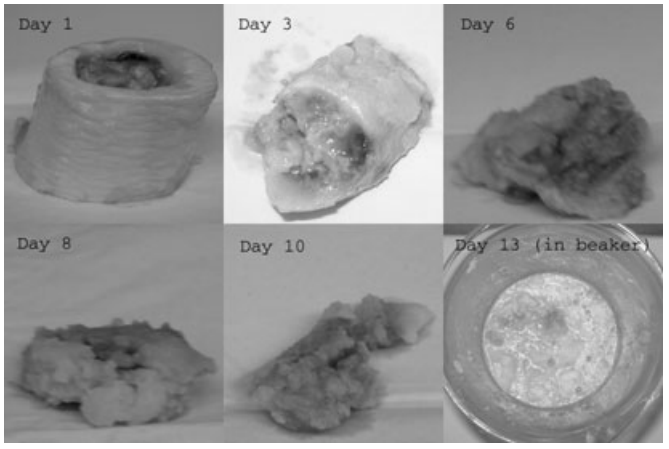

FIG. 1—*pH 1.*

The specimens in the pH 4 solution showed little change until 3 weeks into the study (Fig. 2). (Photographs in picture series may not be the same bone from that solution, and because they were not necessarily photographed on the same day each month, times are approximate.) For the first 3–4 weeks, the loss of tissue was slowly observed with no significant changes seen in the bone. At around 4 months, mold was observed on the surface of the solution and remained throughout the study (this was the only solution where mold was observed). After 1 year, the bones remained in relatively good condition. They were darker in color than the neutral specimens, being a reddish-brown, and the outer surface of the bone had some areas of raised blister-like bumps. The marrow cavity was filled with soft adipocere.

The tap water solution, pH 7, had an expected effect (Fig. 3). Plain tap water (sometimes with heat applied) is often used to macerate remains in forensic contexts and is well-known to effectively remove soft tissue while leaving bones well-preserved (33,34). Over the first few weeks, the tissue slowly began to fall from the bone. By the end of 3 weeks, the absence of tissue on some of the bones was observed, and strands of tissue detached from the bones were observed in the solution. After 1 year, the specimens were in excellent condition. Soft adipocere was present in the marrow cavity, and saw marks on the cut bone surface were still visible.

The pH 10 solution resulted in the best preservation of all of the samples (Fig. 4). After 4 months, tissue still remained adhering to the bones, and after 7 months, significant pieces of soft tissue remained within the solution. After 1 year, some soft tissue still remained in the solution, and the bone was in good overall condition with cut marks still visible. Hard adipocere was present within the marrow cavity and on the outer surface of the bone. This preservation state and adipocere formation is a somewhat expected result because this is the environment most likely to be conducive to adipocere formation.

The pH 14 NaOH solution had a significant and destructive affect on the bones (Fig. 5). By the third day, significant tissue loss

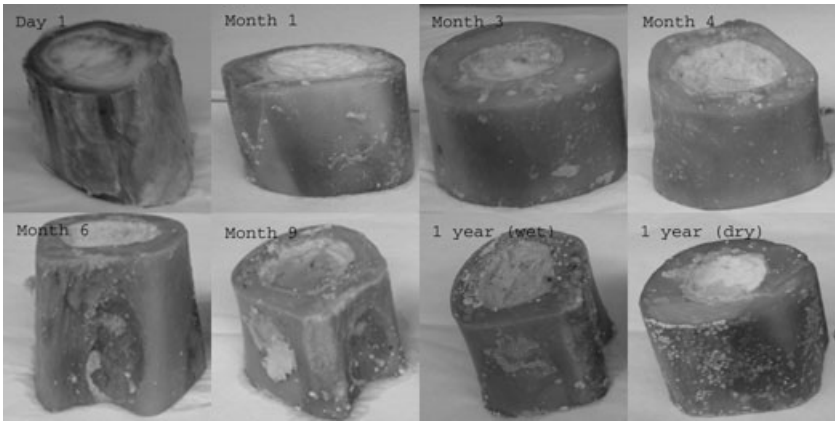

FIG. 2—*pH 4.*

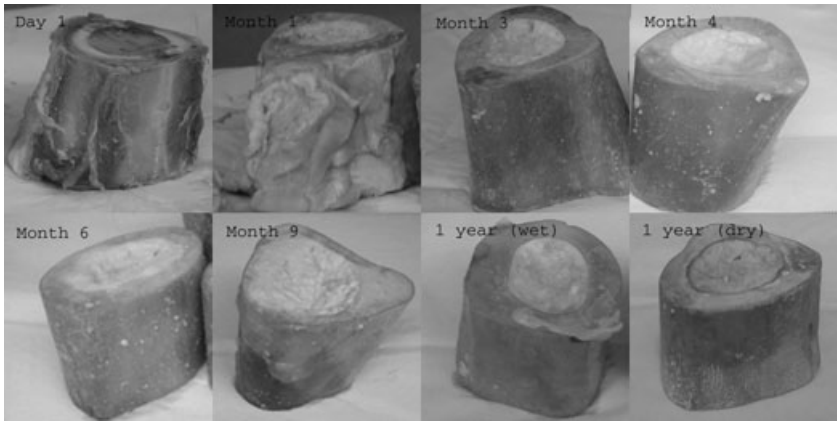

FIG. 3—*pH 7.*

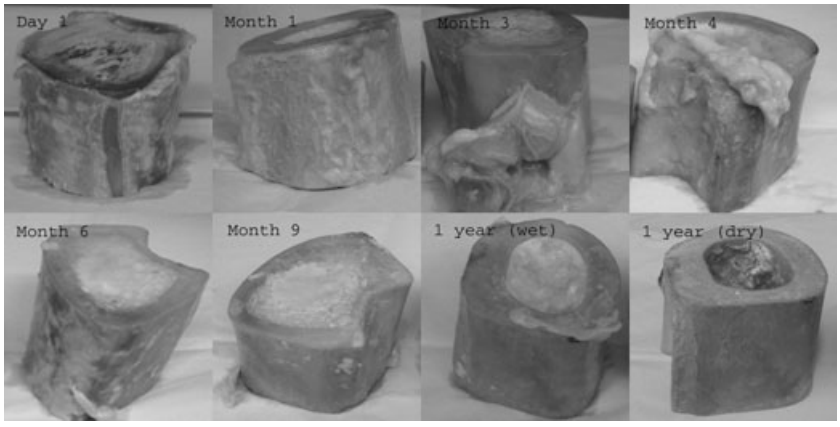

FIG. 4—*pH 10.*

FIG. 5—*pH 14.*

was observed, and by the sixth day, almost all of the soft tissue had been removed from the bone. Effects on the bone were observed by 3 weeks into the study. The outside of the bone surface became waxy in appearance and could easily be marked and/or scraped off, while the interior portion remained hard. By around 4 months, round crystals were observed forming on the surface of the specimens as well as the water line of the beaker. A sample of the substance was removed and examined by a forensic geologist in the FBI Laboratory. The substance was determined to be sodium carbonate hydroxide (aka ''thermonatrite'') that had presumably precipitated out of the solution. The next time water was added to the solution to cover the specimens and adjust the pH level, the thermonatrite dissolved. At *c.* 5 months, pieces of the bones were breaking off in large chunks when handled. After 1 year, the bone was in a state of very poor preservation. The exterior was white and flaky and easily crumbled when handled. Below

the exterior, the bone was more yellow in color, but equally friable. It seems likely that in a forensic context, it may be difficult to recognize the substance as bone.

Discussion and Conclusions

While terrestrial decomposition has been rather extensively studied, little empirical data have been collected on the decomposition of human remains in aquatic environments, and even less is known about the specific effects on bone. Human remains are occasionally discovered in and recovered from aquatic environments in forensic contexts. Thus, empirical data on decomposition and other postmortem changes in these contexts could be extremely useful in both improving time since death estimates and also in assisting recovery experts thereby potentially increasing the quantity and quality of evidence recovered.

This study examined the effects of varying pH levels on bones in aquatic solutions. All solutions eventually removed and dissolved the soft tissues except for pH 10 where soft tissue was removed from the bone but some remained intact within the solution. Moderate (pH 4 and pH 10) and neutral (pH 7) solutions resulted in adipocere formation, especially within the marrow cavity.

The neutral (pH 7) and moderately basic (pH 10) solutions had little effect on the bone, but all other solutions affected the bone to some degree. Extreme pH levels significantly affected the integrity and physical appearance of the bone, completely dissolving it in the case of pH 1 and degrading it considerably in the case of pH 14. Good to excellent preservation was observed in the solutions of pH 4, pH 7, and pH 10, with the moderately basic (pH 10) solution showing somewhat better preservation than the moderately acidic (pH 4) solution. Given that the range for pH of water in the U.S. is around pH 4.3–pH 10 (30–32), one would therefore expect the pH of the water to have little effect on bone preservation (at least over a period of 1 year or less). More information on the effects of pH levels on fully fleshed remains would be needed to improve estimates of time since death, but the results observed here may be useful in making statements regarding time since skeletonization.

While this study was rather small scale and included pH extremes unlikely to be encountered in forensic contexts, it serves as one of the first controlled studies of its kind. We hope that our results will prompt larger empirical studies to be conducted including the use of, for example, larger biological specimens, less extreme pH levels, and varying temperature and salinity.

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Additional information—reprints not available from author:
Angi M. Christensen, Ph.D.
Federal Bureau of Investigation
Laboratory Division
Forensic Anthropology Program
2501 Investigation Parkway
Quantico, VA 22135
E-mail: angi.christensen@ic.fbi.gov