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Effects of Sulphuric Acid on the Histomorphometry of Human Skeletal Remains

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ABSTRACT

Introduction: it is crucial to study the impact of acid on human bones to guide forensic investigations. Hence, the aim of the study was to evaluate the effects sulphuric acid has on skeletal human remains.

Methods: 50 fragments of human femur from the mid-shaft region divided into 10 groups consisting of 5 fragments each were used for the study. They were immersed in different concentrations of 50%, 70%, and 98% of sulphuric acid at different time intervals of 5, 10 and 15 minutes. Modified Frost's Manual method was used for histological preparation of bones. The histomorphometric parameters analysed were the mean haversian canal area (MHCA), mean haversian canal diameter (MHCD), primary osteons (Os-P), secondary osteons (Os-S), osteon fragments (Os-F) and number of non haversian canals (NHC).

Results: The Quantitative analysis showed that the MHCA, MHCD and NHC were not significantly different in the control and groups exposed to acid ($p>0.05$). However, Os-P and Os-F differ significantly ($p>0.05$). The mean primary osteons for the control group was 14.00 while the bone fragments immersed in 98% sulphuric acid for 15 minutes was 3.20. The Qualitative analysis showed that the bone fragments exposed to acid when compared to the control had wide distortions of osteons.

Conclusion: According to these findings, sulphuric acid can have a negative impact on the histomorphometry of skeletal remains.

Keywords: Femur; Humans; Histomorphometry; Sulphuric acid effects; Osteons, Haversian canal.

Introduction

Forensic science plays a crucial role in the resolution of crime, especially such crimes that result in the death of a person. In most crime scenes however, the only piece of evidence available could be the bones of the victim, and this brings the expert opinion of the forensic anthropologist to limelight[1]. The effect of corrosive substances purposefully applied to remains in order to disguise their potential for identification or the immersion of whole bodies to dissolve them as completely as possible, as has been attempted in some criminal cases, is one topic of forensic research[2]. Moreover determining whether a bone was in contact with an acidic solution could be a daunting task for the forensic anthropologists[3].

Different ways can be utilized to obstruct the identification of the corpse or to destroy it in frequent forensic settings. Such activities eliminate proof that a crime has been committed, as well as evidence tying the victim to the crime. Burial, dismemberment, and burning are common methods for disposing of a homicide victim's body. Another option worth considering, particularly in the context of organized crime, is attempting to decompose a corpse using excessively acidic or alkaline liquids, resulting in the progressive breakdown of the tissues. There have been reports that such techniques have occurred, and it is clear that such cases would provide a significant challenge for investigators and forensic pathologists[4].

In a research done by Pokines *et al*., 2016[2], it was stated that corrosive substances could harm bone health by causing dissolution, breakdown, and loss of integrity. As with the corrosion and destruction of bone in acid, these effects can accumulate over time. Previous studies that have explored the application of caustic chemicals for obscuring identity have primarily focused on human teeth, as teeth are widely recognized to be the most enduring skeletal element useful for forensic identification.[5, 2, 6] Oghenemavwe *et al*., 2022[7] also reported that extreme soil pH could cause the weathering of bone, which can distort histomorphometry.

Studies of caustics reacting with bone, hair, fingernails, or soft tissue have been valuable in providing a wider scope to this field of research. These studies however have not included specimen sizes that are necessarily large enough to approximate what an investigator may expect to come across in a forensic context[4,8-10].

Christensen *et al*., 2011[11] found that with small bone samples placed in acid, only extreme pH levels were significantly destructive to fresh bovine bone, and more moderate pH levels allowed for preservation of bone in good or even excellent condition throughout

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an exposure period of up to one year. Amadasi *et al*., 2015[4] noted a similar effect in their experiment on the examination of cut marks on pig (Sus scrofa) bones submerged in solutions of various pH levels.

Further experiments in the same vein found that only strongly acidic solutions were capable of significant alteration to the chemical structure of bones submerged in solutions of various pH levels for a prolonged period.[12] High *et al*., 2015[13] experimented with modern and archaeological mammal bone and found that the demineralization of bone in the presence of sulphuric acid results in the formation of the weaker phosphoric acid, thereby raising overall pH and creating a buffering effect against further destruction.

Additionally, High *et al*., 2015[13] discovered that when heating their samples in sulphuric acid solution to 80˚C in order to accelerate the process of bone damage, the change in temperature precipitated damage even more rapidly than anticipated. A study was designed to observe the effects of a number of variables upon synthetic carbonated hydroxyapatite (CHA) immersed in hydrochloric acid. In this study, Hankermeyer *et al*., 2002[14] found agitation, a decrease in solution pH, and an increase in solution flow rate, solution temperature, and CHA surface area to be positively correlated with the rate of CHA dissolution.

In some studies of the effects of intentional, prolonged exposure of biological tissue to various caustic substances, Cope *et al*., 2006[15] and 2009[16] found hydrochloric acid to be the most destructive, followed by sulphuric acid, then phosphoric acid, with sodium hydroxide the least destructive.

Hartnett *et al*., 2011[9] conducted similar research into the effects of common household corrosives and reported that hydrochloric acid was most rapidly destructive to hard tissue structures, while sulphuric acid took several days to dissolve both bone and teeth completely. Acidic pH levels was reported to be more destructive to bone than alkaline pH levels[12,9]. There is precedent in both forensic casework and industrial accident reports that an alkali solution can in fact be highly destructive to bone when the application of heat is involved[16,17]. Our study therefore aims to compare the histomorphometric features of the human bone exposed to sulphuric acid and those not exposed to the acid.

Materials and Methods

The femur bone of an adult human cadaver was harvested from the dissecting laboratory of the Department of Anatomy, University of Port Harcourt. Cadavers with good femur bone architecture was carefully selected to avoid poor result presentation.

The femur bones were harvested using a scalpel with the cadaver in supine position on the dissecting table. The skin and soft tissues were completely removed and separated from the skeleton using a scalpel with blade. The bony frame with the remains of muscles was submerged in water and covered in a container for about two weeks to allow for softening of the soft tissues for ease of removal.

The bone samples were then divided into different groups, each weighing about 16g and immersed into concentrations of 98%, 70% and 50% of sulphuric acid. This was obtained by adding sulphuric acid to distilled water to achieve the different concentrations. The immersion was done at different time intervals of 5, 10 and 15 minutes. The remaining samples were kept as controls and left in ambient air as a representative sample of the starting time.

Bone tissue preparation: The modified Frost's method was adopted for tissue preparation[18].

Histomorphometric Parameters of the Femur

Osteon Count (OC): The counting of osteon was done by taking note of the number of haversian system.

Haversian Canal Diameter (HCD): It is the distance measured from one end of the haversian canal to the other, covered within its largest possible circumference.

Haversian Canal Area (HCA): HCA was derived from the diameter of the Haversian canal.

Data Analysis: Photomicrographs were taken using the LEICA ICC50 E microscope and at a magnification level of ×100 and x40. The morphometric analysis of the micrographs for osteon count and metrics was obtained using the Image J software (US National Institute of Health, Bethesda, MD, USA). The data obtained were then analyzed using an inferential and descriptive approach with SPSS version 23.

Results

Table 1. Descriptive Statistics of Histomorphometric Parameters of Femoral cortex (Control)

Table 2. Descriptive Statistics of Histomorphometric Parameters of Femoral cortex Exposed to 98% Concentrated Sulphuric Acid for 15 minutes

Table 3. Independent T Test for Differences in Mean for Control and Group Exposed to 98% Concentration of Sulphuric Acid for 10 Minutes

Table 4. Independent T Test for Differences in Mean for Control and Group Exposed to 70% Concentration of Sulphuric Acid for 10 Minutes

Discussion

This study had investigated the effects of sulphuric acid on the histomorphometry of human skeletal remains using the femoral cortex .Our results demonstrated that exposure to sulphuric acid caused alterations in the bone samples at the histological level. The Qualitative analysis of this study showed that the photomicrographs of the acidic bone fragments when compared to the photomicrographs of the control bone showed wide distortions of osteons especially

Table 1. Descriptive Statistics of Histomorphometric Parameters of Femoral cortex (Control).

Parameters	Sample Size	Mean (µ)	SEM	SD	VAR	RANGE	MIN V	MAX V
MHCA	5.00	75.77	6.71	15.01	225.27	37.52	61.35	98.87
MHCD	5.00	9.91	0.63	1.40	1.97	3.89	8.14	12.03
OSP	5.00	14.00	1.30	2.92	8.50	8.00	10.00	18.00
OSS	5.00	14.80	0.37	0.84	0.70	2.00	14.00	16.00
OSF	5.00	18.00	2.19	4.90	24.00	11.00	13.00	24.00
NHC	5.00	0.20	0.20	0.45	0.20	1.00	0.00	1.00

MHCA = Mean Haversian canal area, MHCD = Mean Haversian canal diameter, OSP = Primary osteon. OSS = Secondary Osteon, OSF = Osteon fragment, NHC = Non-haversian canal, µ=micrometre, SEM=Standard error of mean, SD=Standard deviation, VAR=Variance

Table 2. Descriptive Statistics of Histomorphometric Parameters of Femoral cortex Exposed to 98% Concentrated Sulphuric Acid for 15 minutes.

Parameters	Sample Size	Mean (µ)	SEM	SD	VAR	RANGE	MIN V	MAX V
MHCA	5.00	75.09	6.71	15.01	225.33	37.06	59.66	96.72
MHCD	5.00	11.53	0.58	1.30	1.68	3.44	10.01	13.45
OSP	5.00	3.20	1.59	3.56	12.70	7.00	0.00	7.00
OSS	5.00	9.60	2.44	5.46	29.80	13.00	4.00	17.00
OSF	5.00	5.40	1.29	2.88	8.30	6.00	3.00	9.00
NHC	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

MHCA = Mean Haversian canal area, MHCD = Mean Haversian canal diameter, OSP = Primary osteon. OSS = Secondary Osteon, OSF = Osteon fragment, NHC = non-haversian canal, µ=micrometre, SEM=Standard error of mean, SD=Standard deviation, VAR=Variance

Table 3. Independent T Test for Differences in Mean for Control and Group Exposed to 98% Concentration of Sulphuric Acid for 10 Minutes.

Parameters	T Score	Critical T Value	P Value
MHCA	-1.52	2.31	0.17
MHCD	-1.26	2.31	0.24
OSP	8.63	2.31	0.00*
OSS	3.09	2.31	0.01*
OSF	5.85	2.31	0.00*
NHC	1.00	2.31	0.35

*significant

Table 4. Independent T Test for Differences in Mean for Control and Group Exposed to 70% Concentration of Sulphuric Acid for 10 Minutes.

Parameters	T Score	Critical T Value	P Value
MHCA	0.45	2.31	0.67
MHCD	0.56	2.31	0.59
OSP	1.12	2.31	0.29
OSS	4.29	2.31	0.00*
OSF	4.51	2.31	0.00*
NHC	1.00	2.31	0.35

*significant

those that where immersed for a prolonged time and with the highest concentrations of the acid which was about 98% as shown in Figure 1. This finding is in consent with the existing literature as stated by Harnet *et al*., 2011[17] that acids at higher concentrations causes corrosive changes in bones.

Again, the bone fragments immersed in sulphuric acid showed some kind of washing effects as shown in Figure 2 and 3, evidenced by the fact that most of the primary and secondary osteons were poorly distinguished. Some of the haversian canals were highly obliterated as well, especially for the samples exposed to the acid (Fig.2 and 3).

These differences shown were in contrast to those of the control bone whose osteons were well visualized, had clear osteon borders and their interstitial lamella was well defined. In addition, their concentric lamella as well as the haversian canals were well visualized and without any distortion (Fig.1 A). Our investigations suggest that the acid caused the smearing effects, wide distortions of the osteons and haversian canal obliteration in some of the samples.

Amadasi *et al*., 2015[4] also postulated that the highest concentration of acid gave the most destructive effects on the histomorphometry of bones. In addition, samples treated with higher concentrations also led to the massive alterations of the morphological features. This previous study also showed that even without macroscopic alterations, the osteon structure observed under light microscopy was severely deteriorated by acids which varies by the concentration, duration of exposure and as well the type of acid used.

The Quantitative analysis of this research showed marked decline in the number of primary and secondary osteons as well as the osteon fragments

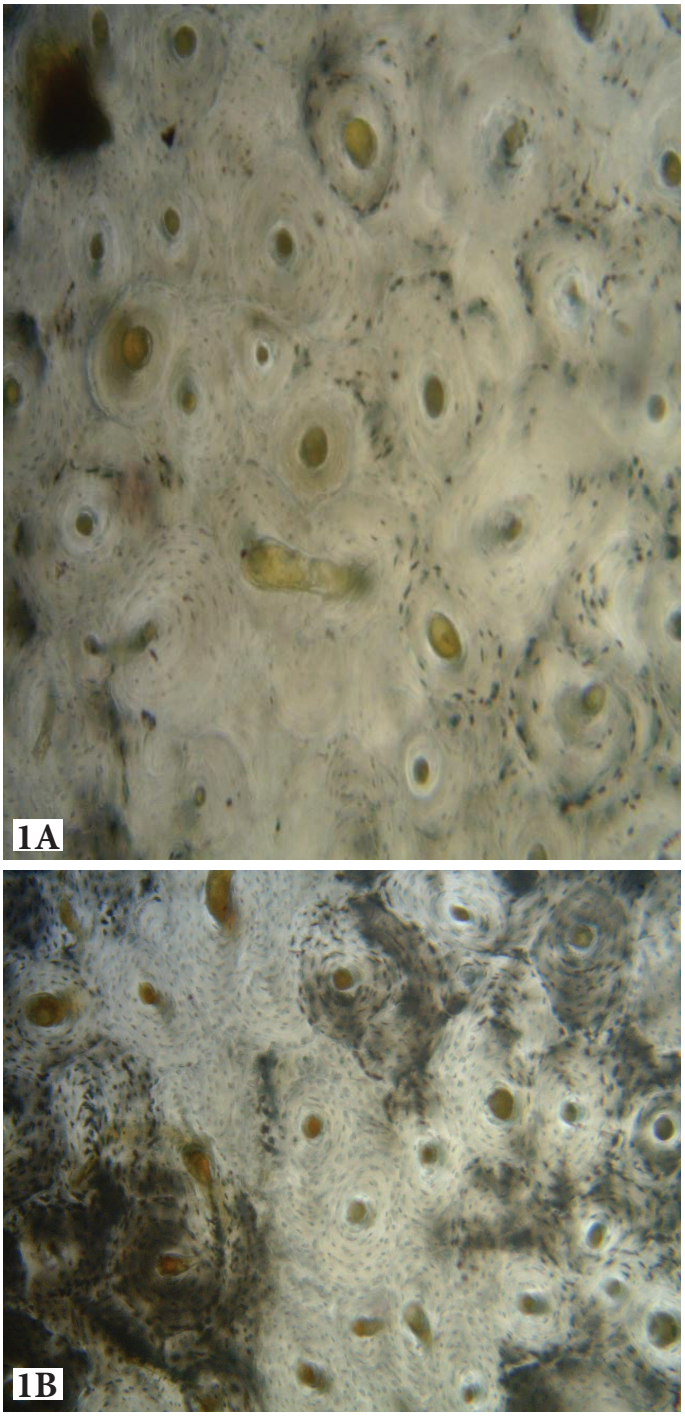

Figure 1. Midshaft of human bone tissue. X100 (A- Photomicrograph of the human femoral cortex not exposed to sulphuric acid, B- Photomicrograph of the human femoral cortex immersed in 98% Sulphuric acid for 5minuites).

when the bone samples were exposed to 98% sulphuric acid when compared to the control group (Table 1 and 2). The mean Haversian canal diameter (MHCD) did increase slightly under 98% sulphuric acid than the control at values of 11.53µ and 9.91µ respectively (Table 1 and 2). The numerical differences in the mean Haversian canal area (MHCA) were not distinct. The non-haversian canals (NHC) were completely lost when exposed to the acid (Table 2). This buttresses

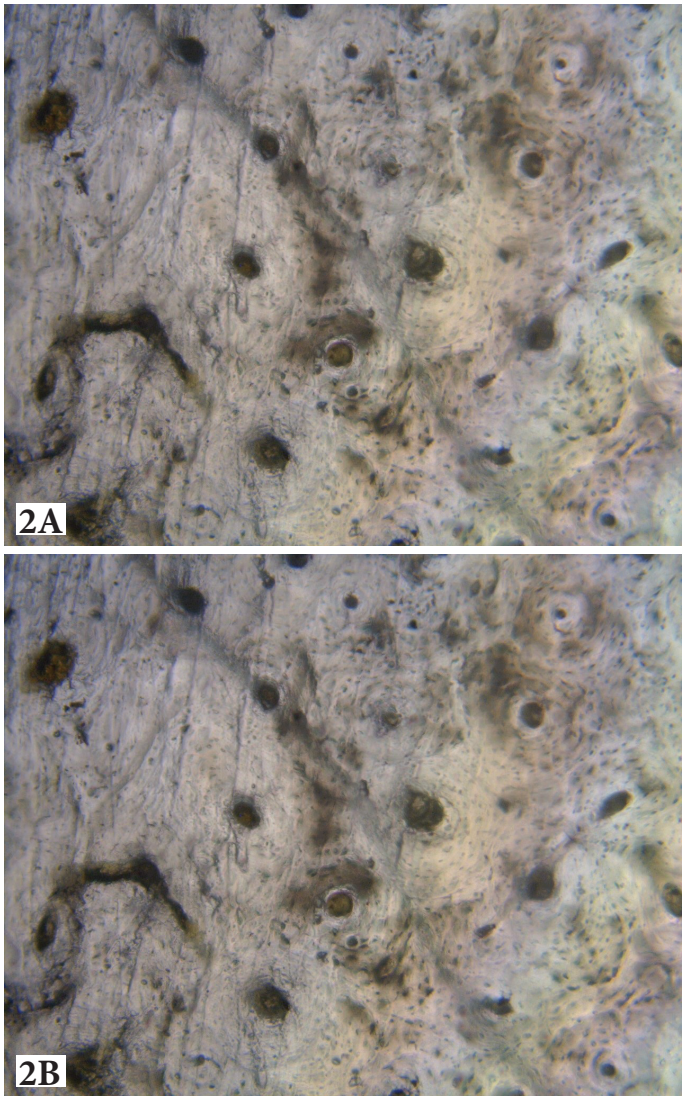

Figure 2. Photomicrograph of the human femoral cortex immersed in 70% Sulphuric acid for 10 minuites. (A-X100, B-X40).

the effect of the acid on the histomorphometry of the femoral cortex being that non-haversian canals were ab initio few in quantity as shown in table 1 of the control group. Further statistical analysis to test for variation between the control group and those exposed to 98% sulphuric acid showed a statistically significant ($p \leq 0.05$) variation in the primary and secondary osteons as well as the osteon fragments (Table 3). The variation in the mean haversian canal area and diameter were not statistically significant ($p \geq 0.05$) (Table 3). This shows that the effects of the acid were notably seen in the osteon count. Osteon number depreciated markedly when the bones were exposed to the acid. At lower concentrations of the acid (70%), statistically significant ($p \leq 0.05$) variation between control and those exposed was seen only in the primary and secondary osteons (Table 4). This shows that more distortion of the bone histomorphometry occurred when exposed to higher acid concentrations and at longer duration.

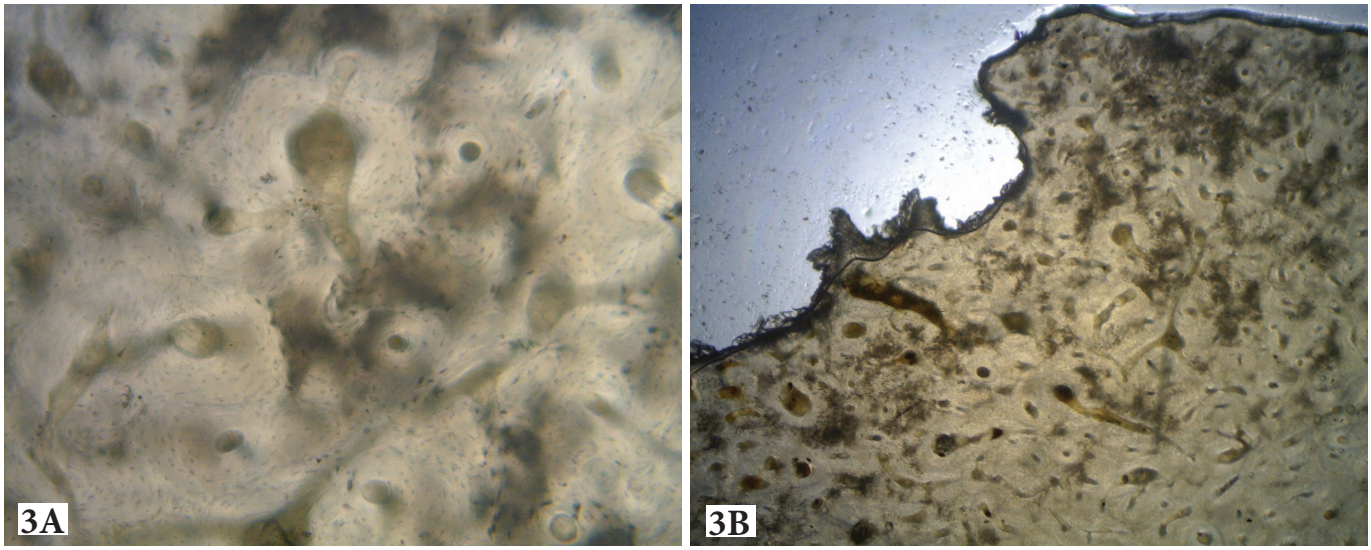

Figure 3. Photomicrograph of the human femoral cortex immersed in 98% Sulphuric acid for 15 minutes (A -X100, B-X40).

Conclusion

According to these findings, sulphuric acid can have a negative impact on bones, leading to increased rates of deterioration and distortion. The quantitative and qualitative data generated by this study is extremely useful when a forensic case shows evidence of chemical modification.

Ethical Approval

The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research[19].

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Mini Curriculum and Author's Contribution

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