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Temperature Related Alterations to Mineral Levels and Crystalline Structure in Porcine Long Bone: Intense Heat vs. Open Flame

Caighley Logan, Suzzanne McColl

Abstract—The outcome of fire related fatalities, along with other research, has found fires can have a detrimental effect to the mineral and crystalline structures within bone. This study focused on the mineral and crystalline structures within porcine bone samples to analyse the changes caused, with the intent of effectively 'reverse engineering' the data collected from burned bone samples to discover what may have happened. Using Fourier Transform Infrared (FTIR), and X-Ray Fluorescence (XRF), the data were collected from a controlled source of intense heat (muffle furnace) and an open fire, based in a living room setting in a standard size shipping container (2.5 m x 2.4 m) of a similar temperature with a known ignition source, a gasoline lighter. This approach is to analyse the changes to the samples and how the changes differ depending on the heat source. Results have found significant differences in the levels of remaining minerals for each type of heat/burning ($p < 0.001$), particularly Phosphorus and Calcium, this also includes notable additions of absorbed elements and minerals from the surrounding materials, i.e., Cerium (Ce), Bromine (Br) and Neodymium (Nd). The analysis techniques included provide validated results in conjunction with previous studies.

Keywords—Forensic anthropology, thermal alterations, porcine bone, FTIR, XRF.

I. INTRODUCTION

THROUGHOUT the country, environmental fires, industrial fires and housefires are a common occurrence, however those resulting in fatalities are becoming more frequent. In the year ending June 2022, there was an estimated 275 fire related fatalities in the UK [4], an increase of around 10% since the previous year with an estimated 251 fatalities. Fire related fatalities are those that would not have occurred if a fire had not happened, this also includes inconclusive cause of death when a fire has occurred [4]. Fig. 1 shows a collection of fire induced fatalities over a 10-year period, including the specified abnormal data collected from the Grenfell Tower fire.

Fire severity refers to the output of a fire's energy, level of destruction that may be caused by a fire and its effects on the surrounding environment, i.e., the more severe a fire, the higher the loss of organic and inorganic materials [12]. The severity of a fire is due to the ignition source and its volume, however, spreading fires are often in abundance of ignition source as the flames spread to various flammable materials, for example, regarding Fig. 1, fires situated in residential buildings will have a myriad of ignition sources such as furniture fabrics, wooden structures, electrical equipment etc.

Classes and types of fire are often split in a multitude of ways, most commonly known is the classes of fire in which the split is based on the ignition source, such as Class A referring to flammable wood or Class E referring to electrical equipment [8]. However, there are also types of fire based on the way a fire may act, i.e., how it burns, this includes controlled fires, uncontrolled fires, wildfires, and structural building fires. This schematic of fires has been developed in regard to the manufacturing of clothing for firefighters and thus an understanding of the hazards for each type of fire. Controlled fires involve multiple subcategories of burning, such as cooking fires, melted metals and others that may be caused by a singular combustible source [21]. Uncontrolled fires refer to a fire that threatens to destroy life, property or natural resources and burns outside of the confines of a firebreak, a prepared space which is designed to prevent a fire from spreading further [16]. Wildfires are an uncontrollable fire in natural environments and are often labelled as such based on their location, for example, bushfires, forest fires or grassfires etc [1]. Structural building fires are those primarily referenced in Fig. 1, mainly occurring in residential, community or commercial based buildings, and hold the greatest amount of variance when considering ignition due to the plethora of flammable items in the aforementioned areas [21].

Due to the increase in fire related fatalities, forensic applications, in recent years, have begun to interpret the thermal alterations to skeletal remains. However, due to the inconsistency of the results that can be obtained through burned skeletal remains [24], there is a multitude of changes that can be analysed. This includes aspects such as changes in colour, changes in levels of porosity or density and the appearance of fractures and splinters. Changes in the levels of minerals and other elements can be affected by a fire's temperature and the ignition source. The changes in minerals can influence the crystalline structure within bone, composed of primarily Phosphorous Ions (PO_4) and Calcium Triphosphate (CO_3), known more commonly as Hydroxyapatite [$(Ca)_{10}(PO_4)_6(OH)_2$]. Studies have found a fundamental change in the crystallinity index of a bone when heated. As the temperature increases, hydroxyapatite effectively transforms into a 'purer' form of itself thus increasing the crystal sizes [20]. The increase in crystal size reduces the porosity of the hydroxyapatite structure as each crystal begins to fill the

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intracrystalline space, however, this does not contribute to the overall porosity of the bone sample as the overall porosity

begins to increase as the temperature rises leaving the bone much weaker and more fragile [20].

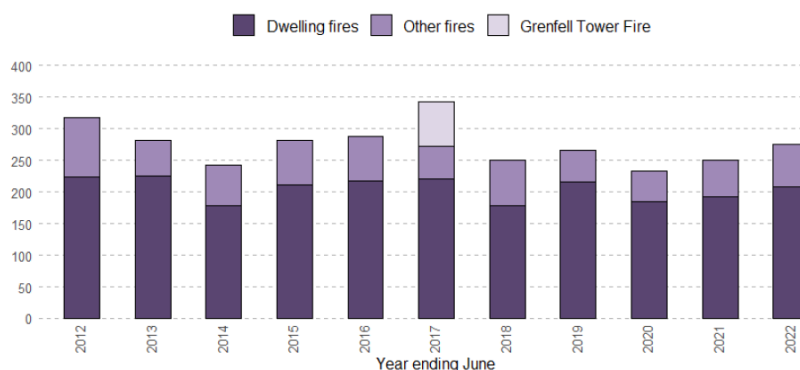


Fig. 1 National statistics total fire related fatalities, England; Year ending June 2012 – June 2022 [4]

During heating or burning, as expected, the majority of minerals within a bone sample decrease, whilst other minerals such as Phosphorus (P) and Calcium (Ca) have been found to increase or remain the same. The presence of new elements within a sample reading has also been observed [19]. This addition of elements is often due to the absorption of surrounding materials whilst the sample is exposed to heat, i.e., burning materials or ignition sources. This has been found in other forensic investigations in which the detection of sharp force trauma on fresh and burned bone found chemical traces of the blades used on both fresh and burned samples [19].

Due to the ethical and legal use of human bone for scientific research, porcine bone is often substituted. Primarily the use of porcine samples focused more on the evaluation of organs or soft tissues as size and functionality were similar to human, however, it has also been found the use of porcine bone can recreate that of human bone in certain conditions. Noted differences in patterns have been observed between human and porcine bone, including such aspects as porosity, elasticity, and hardness [2] however, the knowledge of this can then be implemented if applying the results obtained to that of human based scenarios.

The validity of this research is to contribute to current forensic science analytical techniques and investigations in a medicolegal matter. This research also contributes to the current literature within the subject as there is a current lack of literature within the topic of forensic osteology, particularly with a focus on thermal alterations, or papers that are now outdated by 10+ years.

II. METHOD

Each of the porcine femurs were prepared through maceration. This process involved the bones being frozen for storage until the experiment was ready to take place. The bones were defrosted and boiled in a pan of water with 50 ml of detergent per bone, thus equalling 100 ml. As the size of the bones varied slightly, the amount of water used varied also so to ensure the femurs were completely submerged. The detergent used was a simple non-bio washing detergent to ensure the flesh and other soft tissues or fats were broken down and easily

removed from the bone without causing damage. The remaining flesh was easily removed with a small scalpel and tongs, with great care being taken to ensure no cut marks were made in error or any damage to the integrity of the bone occurred.

The source of intense heat applied to the samples was provided by a muffle furnace. This furnace can reach temperatures of over 1000 °C as it is primarily used for fusing glass/ceramics or decontaminating geological samples [13]. The furnace was set to reach 600 °C. The process of the muffle furnace included a one-hour heating session with the maximum temperature remaining for a chosen set of 3 hours, and a final cooling session over a course of time. The chosen temperature has been known to remove any organic material remaining in the bone material without depleting the structure or integrity of the sample. This temperature was also favoured as studies have found cubic crystallisation formations to occur when heated to higher temperatures whilst the trabecular bone begins to roughen, implying the beginning of the process of recrystallisation [6].



Fig. 2 A photograph of the storage container used for a living room scenario



Fig. 3 A photograph showing the placement of porcine samples in conjunction to the point of ignition

The source of open flame was gained through working with Merseyside Fire Brigade. A shipping container, measuring 2.5 m x 2.4 m x 3 m was filled with various home furnishings to replicate a living room scenario (Fig. 1). The samples were

placed near the ignition source (Fig. 2), ensuring maximum heat damage. The fire was started with the use of a gasoline lighter setting fire to papers in a waste bin; the lighter was also added to the bin for further fuel. The fire burned for an estimated 11 minutes and 32 seconds and reached 603 °C.

Each of the bone samples were analysed post maceration/prior to heating and post heating. This analysis involved the use of FTIR with the Attenuated Total Reflectance (ATR) method and muffle to collect the levels of various minerals and elements within the samples and their concentrations.

III. RESULTS

FTIR-ATR Results

As seen in Fig. 4, 1800 cm^{-1} and below show the levels of Amide I, II and III thus confirming the presence of collagen in the samples. 2500 to 3000 cm^{-1} shows the presence of Phosphorous Ions (PO_4) and Carbon Trioxide (CO_3), confirming the presence of hydroxyapatite and crystallinity. The levels of CO_3 and PO_4 each reach an absorbance level of around 0.03-0.04, further implying the presence of hydroxyapatite [11].

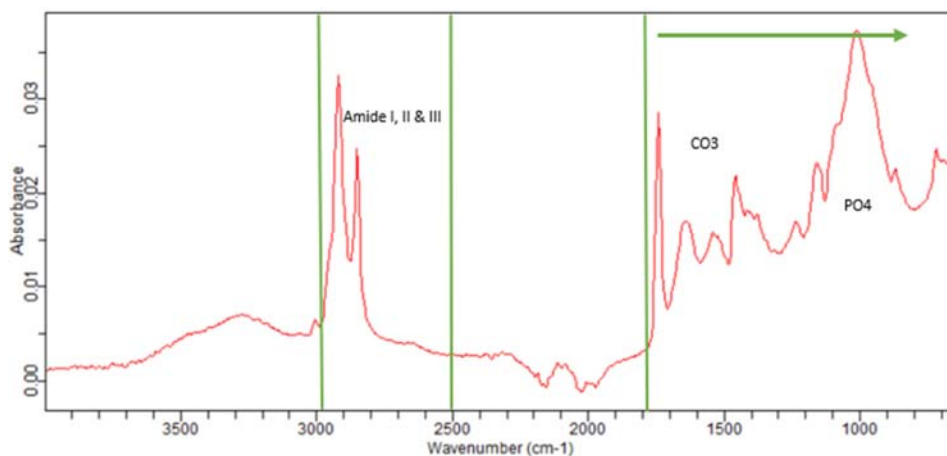


Fig. 4 FTIR-ATR reading of porcine femur control sample

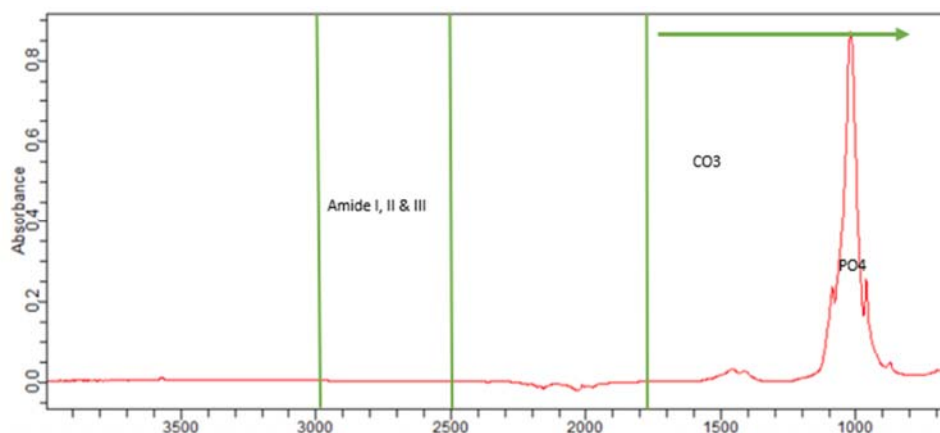


Fig. 5 FTIR-ATR reading of porcine heated to 600 degrees Celsius in muffle furnace

Fig. 5 shows the complete depletion of the levels of Amide I, II and III, due to the intense temperature the bone was exposed to. This result is expected as the loss of collagen coincides with studies that have found bones exposed to temperatures of 600 °C and over lose all organic materials [15]. There is a stronger and more localised peak at 800-1200 cm⁻¹ showing an increase

in the levels of PO₄ and CO₃ therefore an increase in crystallinity and the crystal size. In comparison with Fig. 4, a much stronger absorbance rate of the hydroxyapatite peak is seen. This peak rises x30 the original measurement to 0.9 from 0.03.

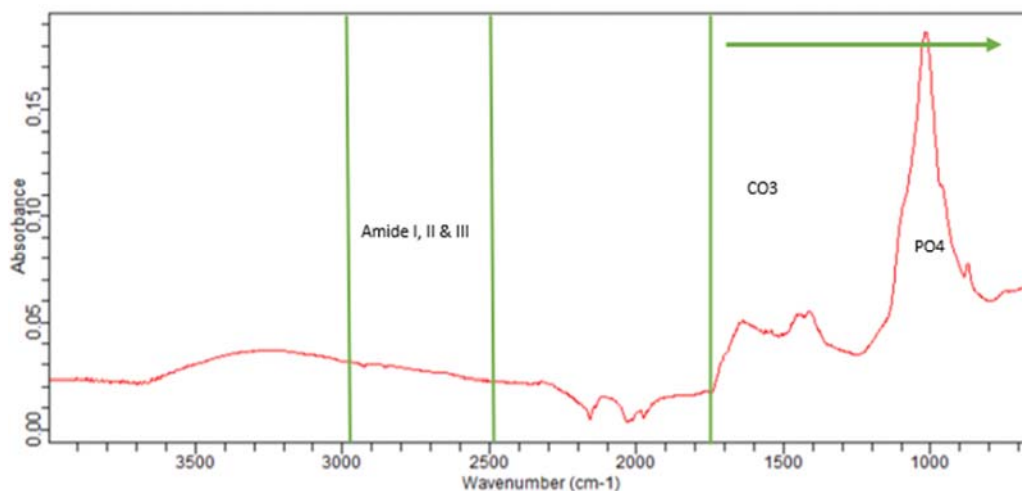


Fig. 6 FTIR-ATR reading of porcine femur samples heated to ~600 degrees Celsius in open flame fire

Element	Control (%)	Open flame	Muffle furnace
Silicon (Si)	0.821	0.417	N/A
Phosphorous (P)	15.238	15.888	15.348
Sulphur (S)	0.204	0.097	0.051
Chlorine (Cl)	0.194	0.18	0.122
Potassium (K)	0.440	0.369	0.322
Calcium (Ca)	82.661	82.696	82.617
Titanium (Ti)	0.032	0.057	0.000
Vanadium (V)	0.000	0.002	N/A
Chromium (Cr)	0.002	N/A	N/A
Manganese (Mn)	0.001	0.002	N/A
Iron (Fe)	0.135	0.071	0.013
Copper (Cu)	0.025	N/A	N/A
Zinc (Zn)	0.094	0.102	0.016
Strontium (Sr)	0.105	0.042	0.056
Zirconium (Zr)	0.001	0.001	0.001
Tin (Sn)	0.050	0.021	0.010
Tellurium (Te)	17.000	0.019	0.008
Samarium (Sm)	0.000	0.000	0.001
Europium (Eu)	0.000	N/A	N/A
Ytterbium (Yb)	0.007	0.006	0.005
Osmium (Os)	0.001	N/A	N/A
Cerium (Ce)	N/A	0.000	N/A
Neodymium (Nd)	N/A	N/A	N/A
Bromine (Br)	N/A	0.03	N/A

Fig. 7 XRF element concentrations of porcine long bone samples; Control, open flame (~600 °C) and muffle furnace (600 °C)

Similar to Fig. 5, Fig. 6 shows a strong loss of Amide I, II and III within the sample, however some remains. This may be due to the presence of various materials in the environment when the burning took place thus reducing the intensity on the sample itself, as opposed to the muffle furnace sample being the only sample in direct heat. In comparison to Fig. 5, Fig. 6 shows the peaks for hydroxyapatite have again increased and have an absorbance recording of 0.19 but this, however, remains less than Fig. 5, again potentially due to the surrounding materials also involved in the burning, or to the placing of the sample in correspondence with the flames.

XRF Results

Fig. 7 shows the XRF results produced from each of the samples, control, muffle furnace (600 °C) and open fire (~603 °C).

Fig. 7 compares the results obtained from the XRF for each of the samples. As expected from the control sample, there are varying minerals and elements in the sample with calcium and phosphorous measuring the highest. However, as the samples have been heated/burned, the levels of many minerals decrease. Contrastingly, levels of calcium remain steady and phosphorous increases. Elements recorded as N/A or 0.000 are included due to their presence on the XRF results even though the low figures represent a miniscule trace of the element. This variance in mineral concentrations can also be seen in Fig. 8.



Fig. 8 XRF results showing the concentrations of minerals in each sample

IV. DISCUSSION

The use of FTIR-ATR allowed for an evaluation of qualitative and quantitative results of the organic and inorganic materials within the bone sample [14]. For the analysis of heat alterations, FTIR has proved useful when determining crystallinity index but, however, it has been found that the results are more applicable from an osteological perspective as opposed to anthropological [23]. As seen in Figs. 4-6, when comparing the results, there is a clear loss of CO₃ and PO₄ within the samples post heating/burning, however, there is a greater loss in the data collected from the muffle furnace. This heating process allowed for a longer exposure to the heat thus resulting in a more intense change of mineral concentrations. The results obtained from the use of an open flame provided similar results with a significant loss of CO₃ and PO₄, however not as much of a loss was recorded, this may be due to the uncontrollable nature of an open flame fire as although care was taken to place the samples nearest to the starting point of the fire, the fires path can easily change unexpectedly. As this fire was conducted in a storage container replicating a living room,

the movement and spreading of the fire was influenced by surrounding materials and therefore not as direct as the heat from a muffle furnace, although the same temperature. The results obtained from FTIR-ATR are in agreement with similar studies, particularly when using the ATR method, i.e., a study conducted by [5] found bone samples that have been defleshed essentially 'hold' the maximum temperatures they had been exposed to as transformations due to heat exposure often occur rapidly and almost instantaneously to exposure. This transformation allowed for heated bone samples to essentially show homogenous heat effects; however, this also depends on the temperature, exposure time and skeletal quality [5]. Nevertheless, the use of FTIR-ATR for the evaluation of mineral content within burned bone samples should be used in conjunction with other analytical techniques as opposed to a sole approach.

As for XRF, the use of this technique provides peaks corresponding to the elemental concentrations within a sample, showing the presence and strength of various minerals [3]. The use of XRF to measure the mineral and elemental concentrations in the samples not only provides information of

what the bone has lost but also what may have been absorbed from external sources. This can be seen in Fig. 7, as there is a clear increase in the levels of phosphorous in each of the samples, more so in the sample affected by the heat of the open flame scenario. Further to this, there is also a consistent level of Calcium within each of the samples, in agreement with the reactions of hydroxyapatite and crystalline structures.

The appearance of various elements is seen in the open flame results but not in the control. The extra elements that have been absorbed are Cerium (Ce) and Bromine (Br). Cerium is a flammable metal among the lanthanides on the periodic table and is often used in welding electrodes and diesel fuel additives to enhance combustion [25]. Bromine is a flame-retardant chemical that is often added to furniture foams and other textiles to prevent flammability [10]. The presence of Ce and Br within the open flame sample can be assumed to have been absorbed from surrounding environment as it burnt. As each of the elements is often used in household items therefore the presence is not unexpected. This finding however, when applied to a medicolegal situation, can allow for a more precise idea of location and environment or potential ignition source when in an investigation. The finding of Ce in particular agrees with this, as the aforementioned ignition source was a gasoline lighter, in which Ce is often found as it is the primary metal in lighter flints [9]. There is also a presence of Neodymium (Ne), which although has been measured at N/A for each sample set, the presence of the element on the results indicates a miniscule trace within the sample. Ne is often used in certain types of glass for the use of lasers [18] and is more than likely a trace reading from the XRF itself as opposed to surrounding environments when heating.

The use of a muffle furnace when applying intense heat to the samples provided a much more controlled and recordable approach when compared to the open flames. The results post-heating of the samples provided more of a basis when comparing the results of less controlled applications of heat.

Placing samples into open flame provided more realistic and uncontrollable results due to the nature of the fire. During the burning process, a potential limitation occurred, in that the samples sizes varied due to the difference in time when creating the samples. The samples were retrieved from the same initial cadaver but however may have ranged in size and volume thus skewing the results. Other factors may also include the process of drying the samples prior to being entered into the muffle furnace, whereas the corresponding samples were simply placed into the open flame environment without drying beforehand.

V. CONCLUSION

This research aimed to identify heat induced alterations in bone, providing an insight to some of the changes that may occur, and the practicality of the analysis techniques used. The application of heat to skeletal remains continues to be a poorly researched topic in the field of forensics, therefore it is important to continue the research and contribute to the literature to aid investigations [22]. The study has found that there are clear differences in the changes that occur to porcine

bone samples depending on the heat source and surrounding materials. Although the samples reacted in various expected ways, the unexpected results are still able to contribute as a cause for validation of the results. Further research will provide a more reliable dataset in regard to the results, and also a dependable preparation method eliminating the limitations that have occurred due to sample size and volume.

As for the analysis techniques, the use of FTIR-ATR and XRF continue to be a reliable source of qualitative and quantitative data collection when evaluating bone samples, both prior to and post heating. As seen in the discussion, various studies repeatedly use each of the analysis techniques amongst others to create a reliable dataset, however further research may also find other more applicable analysis techniques that will further the results found. For instance, the use of Raman Spectroscopy allows for a non-invasive investigation into the chemical composition of bone tissue and a collection of qualitative analysis of heterotopic bone composition [17]. Further studies have found the combination of Raman Spectroscopy with other infrared analysis, such as FTIR, provides valid and consistent results when applied to skeletal remains, particularly those exposed to 600 °C and above, with an identification of clear biomarkers rendering diagenesis caused by intense heat [15].

Although certain limitations surfaced, further work in this topic will continue to validate the collected results. Nevertheless, when comparing to similar studies, the results are viable and can contribute to current findings. For example, the overall reactions of both datasets are in accordance with that of Gallo et al [7]. The study found the structure and vulnerability of bone when burned varies widely between the type of burning that has been administered and also the quality of the samples included.

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