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Exploring chemical traces of sharp force trauma in burned bone through SEM-EDS: A pilot study

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ABSTRACT

Sharp force trauma (SFT) is a leading cause of homicide-related deaths, frequently involving kitchen knives as weapons. Offenders may attempt to eliminate forensic evidence by burning a corpse, complicating medicolegal investigation. While scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) has successfully detected metallic traces from sharp objects in skin and fresh bone, its efficacy in burned bone is currently unexplored. This study aimed to evaluate the potential of SEM-EDS to detect metallic residues transferred from sharp tools to bone that was subsequently burned, thereby contributing to the development of an identification procedure in forensic investigation. Ten knife strikes were inflicted on fleshed porcine ribs, which were then burned in an electric muffle furnace at 700 °C for two hours. Sharp lesions were analyzed using SEM-EDS to identify transferred chemical elements. Traces of iron and chromium were detected in seven lesions, with silicon identified in two samples. These elements, absent from the control samples, provided robust evidence of transfer from the blade to the bone. The residues appeared as bright spots with either undefined shapes or well-defined particles, located along the lesion edges. This study is the first to confirm the identification of metallic traces from SFT in bone subsequently exposed to heat using SEM-EDS. Despite thermal exposure, residues persist and remain detectable. SEM-EDS analysis is thus a non-destructive, valuable technique for distinguishing heat-induced bone damage from SFT. Further research with a larger sample and a broader range of implements is needed to validate and extend these findings for forensic applications.

1. Introduction

In medicolegal investigations, forensic anthropologists provide essential expertise in reconstructing the circumstances surrounding death. Such investigations require complex analyses of traumatic injuries, particularly in cases involving advanced decay, skeletonization, dismemberment, or even carbonization [1]. Among these injuries, sharp force trauma (SFT) is a leading cause of homicide-related deaths in Europe [2], where gun laws are stringent, and the second most common in the USA [3]. Readily available tools, such as kitchen knives, are often used as weapons during impulsive confrontations [4], and the trunk is the most frequently targeted anatomical region [2,5–7]. The analysis of SFT is challenging due to the difficulty in establishing a direct association between bone lesions and the tool used in the aggression.

The analysis of transferable chemical elements from an implement to bone and soft tissues is a growing source of evidence in forensic science.

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Nevertheless, exogenous tool-related residues may also be present due to contamination from soil, clothing, medical or dental devices, arson accelerants, or particles adhering to a previously used implement [8,9].

The research efforts are focused on sharp and blunt force trauma, as well as high-velocity projectile trauma (gunshot residues) (e.g., [10–12]). Analytical techniques such as X-ray fluorescence spectrometry (XRF), X-ray microfluorescence, attenuated total reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), proton-induced X-ray emission (PIXE), inductively coupled plasma atomic emission spectrometry (ICP-AES), and scanning electron microscopy with energy dispersive X-ray (SEM-EDS), among others, have been explored in this context (e.g., [8,10,11,13,14,15]). A wide range of materials has also been tested, including projectiles, metal bars, various types of steel knives, ceramic knives, saws, axes, and scissors (e.g., [10–12,14]).

Bai et al. [13] and Palazzo et al. [16] provided evidence that SEM-EDS can successfully identify metallic residues associated with SFT in skin, demonstrating correspondence between the traces in the lesions and the elemental composition of the tools used. Thompson and Inglis [6], Gibelli et al. [11], and Marginedas et al. [17] extended these analyses to fresh bone sharp lesions, detecting transferred metallic residues. Although the quantity of traces on the kerf floor and at the lesion edges was reported to be reduced and their distribution was highly variable [11]. Vermeij et al. [18] highlighted that both stainless and carbon steel particles are frequently deposited onto the skin or fresh bone during SFT events, supporting SEM-EDS as a technique for detecting the transfer of metallic particles from a specific object to these tissues and for narrowing the possible tool class in forensic investigation. Thus, the value of SEM-EDS for detecting metallic traces in both soft and hard tissues is underscored, providing an important link between lesions and the implements used.

The challenge to establish an association between bone lesions and the tool used in the aggression becomes even more complex when criminals deliberately burn the body to conceal forensic evidence [19–21]. To date, the only study evaluating the potential of SEM-EDS to detect metallic residues in heat-exposed bone was conducted by Amadasi et al. [22], who demonstrated that high-velocity projectile residues persisted in burned bone, albeit at lower levels than in fresh samples.

The present experimental pilot study assessed the potential of SEM-EDS to detect elemental signatures associated with sharp tools in bone subsequently exposed to heat, thereby contributing to the identification of trauma-inflicting implements by analyzing transferred chemical traces. To our knowledge, no study has applied this technique to SFT in burned bones, although this kind of evidence is pivotal in homicide investigation. Moreover, this analysis is becoming relevant for interpreting accidental burn-related deaths in fire accidents and natural disasters, including wildfires, which have significantly risen due to climate change. These events underscore the need for further research to improve the reliability of the analysis and interpretation of burned human remains and associated trauma. Furthermore, a comprehensive understanding of the effects of thermal exposure on bones is essential for advancing methodological approaches in forensic anthropology and bioarcheology.

2. Experimental procedure

2.1. Material

A kitchen knife was acquired from a supermarket to replicate real-world casework in which a sharp tool is readily accessible. A section of the knife blade was analyzed through SEM-EDS to determine its elemental composition. A single knife was used in this pilot study to ensure methodological control and isolate the chemical signature of a well-characterized implement, enhancing internal validity.

The bone samples consisted of a rack of 12 ribs from a domestic pig (*Sus scrofa domesticus*, seven months old, weighing approximately 80 kg) obtained from a local butcher. Despite morphological and histological differences, porcine bone is widely employed in forensic research [3], as the use of human samples raises greater ethical concerns. Notwithstanding, there is a consensual awareness that such replacement may influence the results [20]. The soft tissues were preserved to replicate their resistance before the implement reached the bone, as outlined by Ferllini [5] and Cassidy & Curtis [7]. Moreover, a relatively thin layer of soft tissue covers the thoracic region, making it one of the first anatomical areas exposed during a fire or burn event. Consequently, it is more susceptible to thermal destruction than other anatomical regions [23].

2.2. Bone sharp lesions

All lesions were inflicted by the same researcher using a guillotine (Fig. 1) with an attached knife, controlling the movement of the object and its contact with the sample to ensure the reproducibility of the process [23,24]. The linear downward motion allowed this device's calibration to deliver a work (W) of approximately 29 J at the moment of impact, consistent with the value used by Waltenberger and Schutkowski [23]. The impact angle was 90°, ensuring this variable was not responsible for any differences in the resulting lesions [25].

A total of 10 knife strikes were inflicted on fleshed ribs. In addition, one fresh and one burned rib were kept intact as control samples to evaluate potential environmental contamination.

2.3. Bone heat exposure

Burning experiments were conducted in an electric muffle furnace (Heraeus KR 260 E) set to 700°C for two hours. This temperature was selected as it is described as the maximum temperature reached in an open-air fire or a domestic fire [23]. However, under extreme burning conditions, such as wildfires, cremation or arson involving accelerants, higher temperatures may be attained [26]. After heating, the bone samples were cooled to room temperature before being removed from the muffle.

2.4. Morphological and elemental analysis

The burned bone sharp lesions were examined using a JEOL JMS-5400 scanning electron microscope, coupled with a Bruker XFlash Detector 610 Mini and Bruker ESPRIT Compact software for X-ray energy dispersive spectrometry, and captured images for elemental analysis. The samples were cut using an NSK diamond disc to allow mounting onto the SEM-EDS stub. To prevent contamination, the lesions were protected during the cutting process, which was performed on the area of bone farthest from the lesions. Preliminary cutting tests were

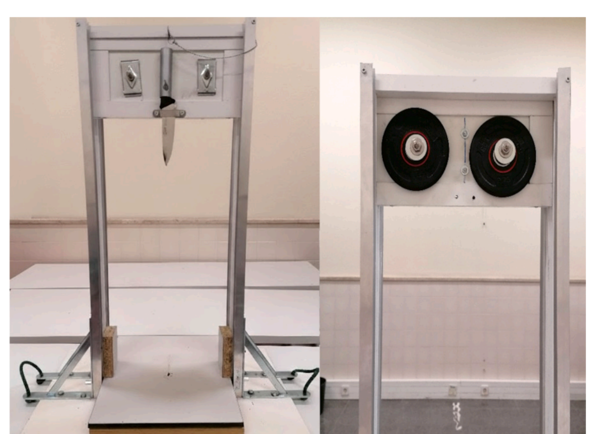

Fig. 1. Guillotine: anterior view with an attached knife, and posterior view with weights for calibration.

performed to assess potential contamination prior to processing the burned bone samples.

A qualitative approach was adopted to identify the chemical elements present in the samples. The quantitative analysis was discarded due to the irregular nature of the bone surface, which would compromise the accuracy of the elements' quantification. Indeed, the irregular morphology of the cutmarks, resulting from bone splintering and raised margins, can cause significant deviations in the X-ray trajectory toward the detector, rendering the extrapolated quantitative results useless [27,28]. Furthermore, when the particles were morphologically distinguishable, they were counted. The samples were not coated with gold or carbon to avoid contamination. Thus, to enhance conductivity, a copper tape was placed on the underside of the sample. A 20 kV voltage was selected for the elemental analysis.

The analysis was conducted around each sharp lesion, including within it and a surrounding 2 cm margin, accounting for the random distribution of residues transferred to bone, as previously reported by Gibelli et al. [11]. Morphological data and chemical elements were identified using magnifications ranging from 35x to 50x. Subsequently, higher magnifications (100x to 2000x) were employed to detect potential metallic traces.

3. Results

3.1. Control samples

Control samples (Fig. 2) were established for the knife and both fresh and burned bone. The elemental composition of the control knife sample included iron (Fe) (72.08%), carbon (C) (15.24%), chromium (Cr) (12.19%), and a minor fraction of silicon (Si) (0.50%). The fresh bone control sample consisted of C, oxygen (O), calcium (Ca), and phosphorus (P). In the burned bone sample, elements such as potassium (K), sodium (Na), magnesium (Mg), and aluminium (Al) were identified, along with O, Ca, and P, also present in the fresh bone sample.

The burned bones exhibited a greyish coloration – 10YR 6/1 [29] – indicative of the inversion phase [20,30], along with a reduction in size compared to fresh bone as reported by Srivastava et al. [20]. Typical heat-induced fractures, as described by Symes et al. [31], were also observed. Thermal exposure resulted in the loss of a sharp lesion in one rib, preventing its localization, while two other ribs exhibited no detectable metallic traces (Table 1).

3.2. Burned bone sharp lesion samples

Table 1 presents the elemental composition determined by SEM-EDS in the sharp lesions. In addition to the elements identified in the burned bone control sample, traces of Fe and Cr were detected in the seven lesions (Figs. 3b–6b). Intriguingly, Si was identified in only two of the samples.

Morphologically, metallic traces on bone can be categorized into two groups: (i) residues exhibiting an undefined shape with no well-defined margins, diffusely distributed across the lesion (Fig. 3) and (ii) traces with distinguishable particles presenting well-defined margins and larger dimensions (Fig.s 4–6). The lesions exhibited three to 22 well-defined particles with diameters ranging from 0.69 to 3.76 µm. Interestingly, all traces were detected in the lesion edges.

4. Discussion

4.1. Control samples

The elemental composition of the control blade's knife sample reflected the typical profile of a steel alloy, primarily consisting of Fe and C, with Cr and Si added to enhance corrosion resistance and blade hardness [13,32].

Fresh and burned bone control samples exhibited the expected

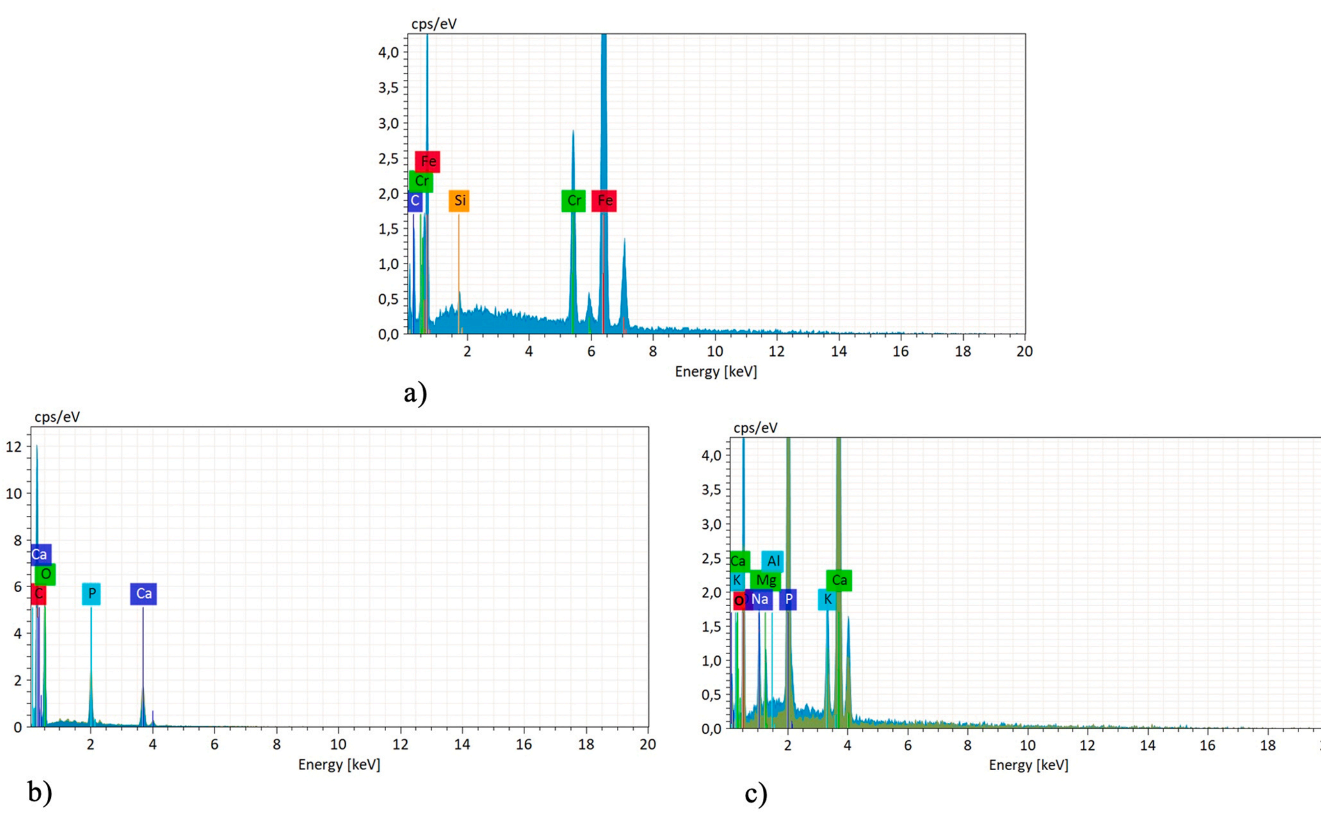

Fig. 2. SEM-EDS spectrum from a blade's knife section (a), fresh bone control sample SEM-EDS spectrum (b), and burned bone control sample SEM-EDS spectrum (c). The x-axis represents the energy (keV), and the y-axis indicates the abundance (cps/eV).

Table 1
Elemental composition of bone sharp lesion samples (1–9) after burning.

Samples	Elemental composition											Number of particles per lesion
	C	O	Na	Mg	Al	Si	P	K	Ca	Cr	Fe	
Knife	X					X				X	X	-
Fresh bone (control)	X	X					X		X			-
Heat-exposed bone (control)		X	X	X	X		X	X	X			-
1							X			**X**	**X**	4
2							X		X	**X**	**X**	20
3		X	X				X		X	**X**	**X**	22
4		X							X	**X**	**X**	Diffuse distribution
5		X							X	**X**	**X**	3
6		X	X	X	X	**X**	X	X	X	**X**	**X**	8
7		X				**X**	X		X	**X**	**X**	13
8		X	X	X	X		X	X	X			0
9		X	X				X	X	X			0

Bold X indicate the presence of metallic traces in the bone sharp lesions

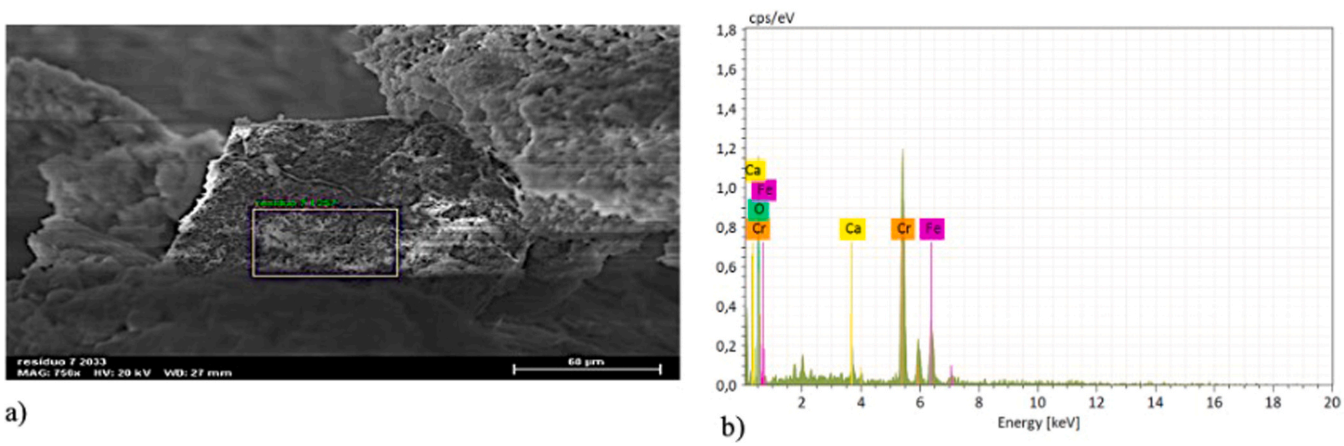

Fig. 3. Metallic traces on the burned bone sharp lesion (sample 4): (a) SEM-EDS micrograph showing the diffuse distribution of the metallic traces and (b) SEM-EDS spectrum.

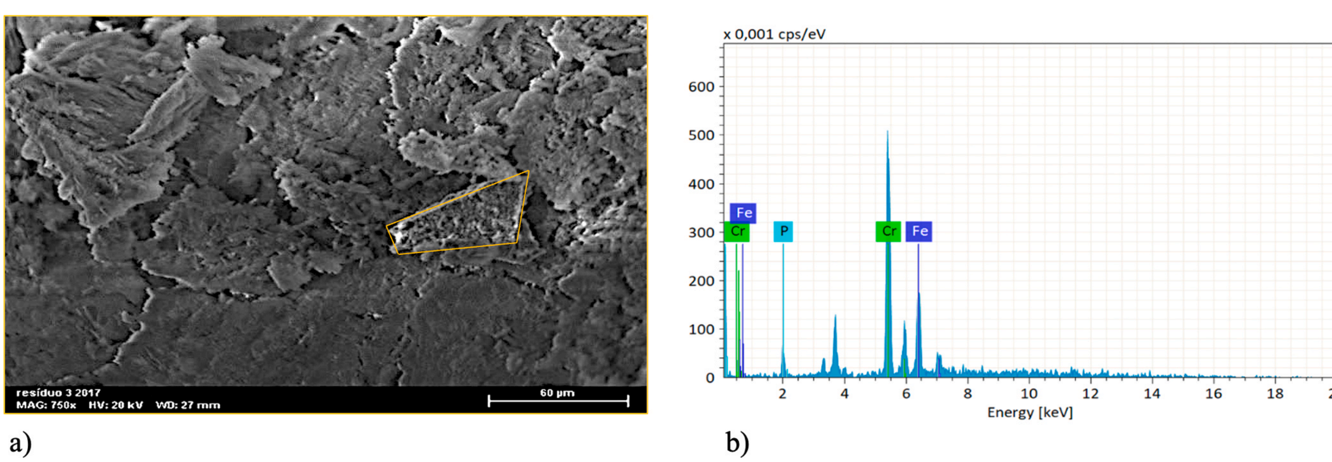

Fig. 4. Metallic traces on the burned bone sharp lesion (sample 1): (a) SEM-EDS micrograph revealing spots of trace particles and their delimitation, and (b) SEM-EDS spectrum.

chemical elements present in the organic and inorganic constituents of bone tissue [33–36]. For instance, Na, Mg, Al, and K are associated with ionic substitution at the level of the calcium phosphate matrix and are common in fresh and burned bone [35,36]. The presence of Na and K in the burned bone can be attributed to the post-mortem fluids and hemolysis, as the samples were burned with soft tissue [37]. The noticed increase of Na, K, and Mg with rising temperature is relative and results from the depletion of other elements [33]. The absence of C in the samples was expected, as organic material breaks down into carbon monoxide and carbon dioxide, which evaporate completely when heated to 500 °C. Moreover, carbonates undergo complete degradation after prolonged exposure to temperatures above 500 °C or upon reaching 1100 °C [34]. Concerning the O, which is found in water, organic constituents, and bone structural carbonates, its amount changes non-linearly with heat exposure, since dioxygen acts as an oxidant in bone combustion. At temperatures above 600 °C, carbonates begin to

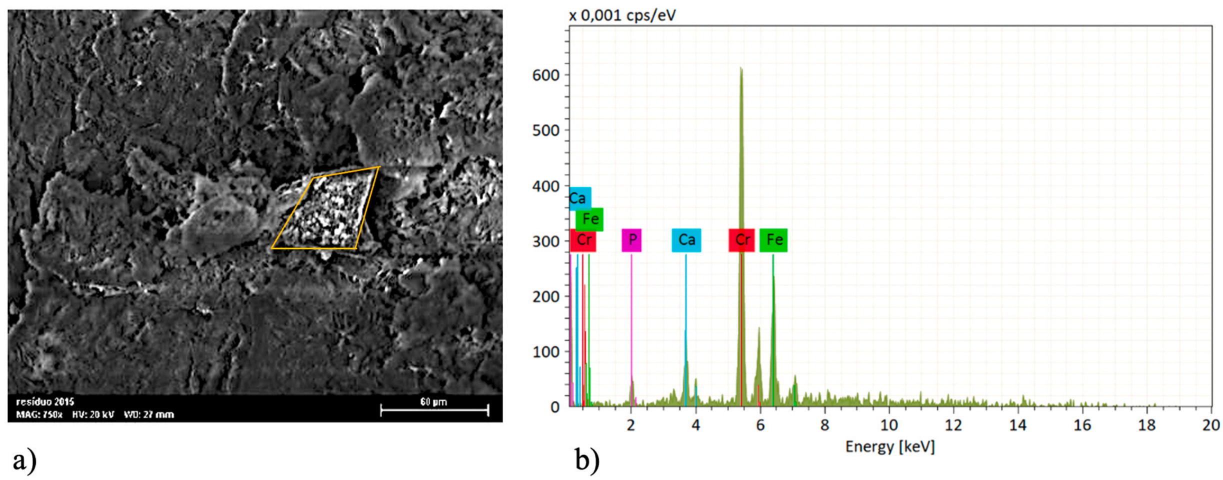

Fig. 5. Metallic traces on the burned bone sharp lesion (sample 2): (a) SEM-EDS micrograph exhibiting spots of trace particles and their delimitation, and (b) SEM-EDS spectrum.

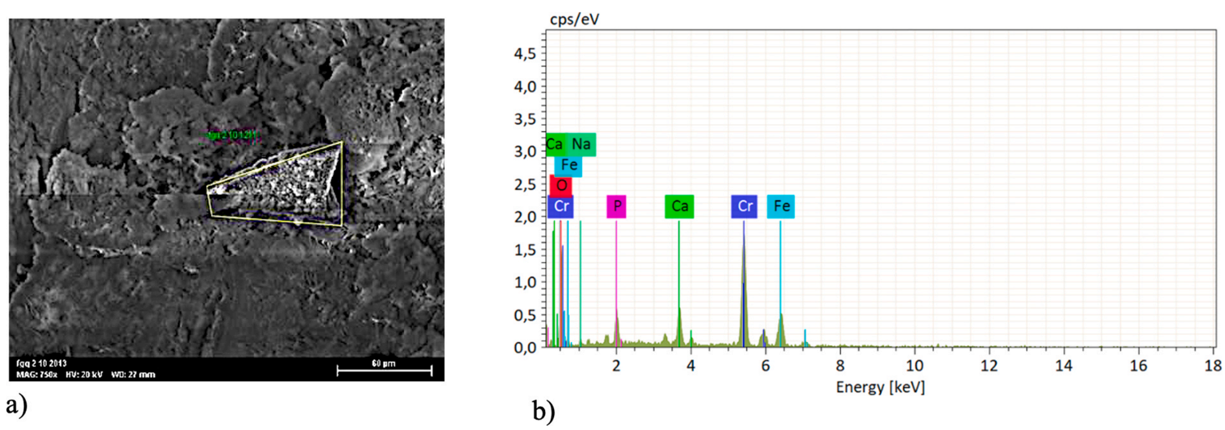

Fig. 6. Metallic traces on the burned bone sharp lesion (sample 3): (a) SEM-EDS micrograph showing spots of trace particles and their delimitation, and (b) SEM-EDS spectrum.

evaporate, reducing the amount of O. However, above 700 ºC, O levels increase due to the formation of calcium oxide [34]. Ca and P exhibit only minor losses at 700 °C and no significant changes at higher temperatures, indicating that the mainly inorganic constituents of bone are thermally stable even under extreme heat exposure [33].

Moreover, it should be noted that heat-induced changes in bone microstructure may influence the retention of residues, as microstructural restructuring affects whether metals penetrate, bind to, or remain on the bone surface. As temperature rises, particularly between 500 °C and 800 °C, progressive loss of organic constituents increases porosity, creating pathways for surface metallic particles to migrate into deeper bone layers [14]. Furthermore, heat promotes micro- and macro-morphological changes (e.g., shrinkage, warping), especially above 700 °C, which may either obscure metallic residues or expose concealed ones [14,19,38]. These restructuring movements can hinder detection when surface-sensitive techniques such as XRF are used [14]. In contrast, SEM-EDS is not restricted to the external bone surface; its imaging and analytical capacity allow assessment of both the surface and the internal areas of the lesion, providing greater potential for detecting embedded particles.

4.2. Bone sharp lesion elemental analysis

SEM-EDS analysis confirmed that bright spots observed in the lesions contained Fe and Cr in seven samples. (Figs. 3–6). In addition, Si was detected in two samples. These metallic elements were absent in the inorganic composition of both fresh and burned bone control samples, providing robust evidence of residues transfer from the knife to the bone. Additional elements associated with the bone matrix were identified, varying according to the analyzed area and the definition of the bright spots [35,37].

Previous research has demonstrated that SEM-EDS can detect gunshot residues from high-velocity projectiles in both fresh and burned bone, although thermal exposure reduces trace abundance [22]. Similarly, transfer of metallic particles to skin and fresh bone in sharp and blunt force trauma was also observed [11,13,16,18]. In this study, the detection of transferred knife residues on bone after subsequent burning aligns with earlier observations in skin [13,16] and fresh bone [6,11,17], supporting the persistence of tool-related residues across different tissues and post-mortem conditions. Consistent with Gibelli et al. [11], who analyzed fresh bone sharp lesions, the quantity of metallic traces detected was reduced and their spatial distribution highly variable, reinforcing the need for meticulous and systematic search protocols. Additionally, Vermeij et al. [18], analyzing trace transfer onto the skin and fresh bone, emphasized that SEM-EDS can refine the potential weapon class or confirm its involvement in aggressions when a questioned tool is available.

In this context, the present study extends existing knowledge by demonstrating that metallic residues associated with SFT can also persist in bone subsequently exposed to heat. Whereas previous research has focused either on fresh bone and soft tissues or on gunshot wounds in heat-exposed bone, the detection of Fe, Cr, and Si in burned bone sharp lesions bridges this gap and corroborates that thermal exposure does not preclude trace survival, contributing valuable evidence to medicolegal investigation.

Together, these findings support SEM-EDS as a potential tool for identifying metallic residue transfer under challenging post-mortem conditions, while also highlighting its current limitations for precise tool identification. In fact, elemental concentrations below the EDS detection threshold may hinder accurate determination and complicate differentiation of steel types, as many alloys contain trace elements such as vanadium, molybdenum, tungsten, silicon, manganese, and nickel [18], which may be critical for distinguishing between different steel compositions.

4.3. Morphology and distribution

The metallic traces transferred from the knife exhibited higher conductivity, appearing as brighter spots in lesions under SEM-EDS analysis. Morphologically, these residues were either undefined, with diffuse margins, or trace particles with well-defined margins. These findings partially align with Gibelli et al. [11], who also reported highly heterogeneous metallic trace sizes and a low number of residues in fresh bone sharp lesions; however, in contrast to their study, residues exhibited clustering or a diffuse distribution.

Regarding spatial distribution, traces were exclusively confined to the lesion edges. According to Gibelli et al. [11], the location of residues depends on the tool used and can be observed on the kerf floor, edges, walls, and surface. However, on steel non-serrated knives, traces are most frequently identified on the kerf floor and are scarce at the lesion edges. Marginedas et al. [17] identified traces on the kerf floor, walls, and lesion edges of fresh bone. Nevertheless, due to the bone microstructure heat-induced restructuring, no direct comparison can be made with these studies. Thus, this study is the first to show the spatial distribution of metallic traces from SFT in bones subsequently exposed to heat.

Our results indicate that residues persist even under extreme thermal conditions, which are likely encountered in an open-air or domestic fire, challenging the assumption that heat exposure eradicates all forensic evidence. Although burning is a destructive process, it does not preclude the detection of residues from sharp implements.

The reduced quantity and random dispersion of these traces are not necessarily a consequence of thermal exposure, as similar results have been documented in fresh bones sharp lesions by Gibelli et al. [11]. Additionally, Bai et al. [13] and Palazzo et al. [16] reported significant metallic transfer at the skin level. Therefore, we suggest that most metallic traces remain embedded in soft tissues, with only minor amounts reaching the bone. This assumption requires further validation but carries significant forensic implications, as the extent of residues transfer may depend on the presence and condition of overlying soft tissues at the time of trauma and body recovery.

4.4. Study limitations

Limitations include a small sample size, the use of a single knife, and the application of controlled mechanical strikes and thermal exposure rather than dynamic body-weapon interaction or open-fire conditions to fully simulate real-world forensic casework. Furthermore, although porcine bone is widely used as a human proxy, its greater brittleness may favor retention of residues within the lesion [3], potentially resulting in higher levels of metallic traces than in human bone and thus influencing the results. Accordingly, faunal bone results are not directly transferable to humans, given interspecies variation in heat-induced changes [20], which may limit the practical applicability of such findings.

The single-knife use fulfilled the aim of this study but does not allow the assessment of inter-tool variability and restricts broader interpretation and forensic applicability. Further research using larger samples and a wider range of sharp tools is needed to expand and validate these findings prior to human bone testing.

Finally, this experimental pilot study is further limited by the lack of pre- and post-burning comparative analyses, as the absence of baseline data from unburned samples precludes assessment of thermal effects on residue loss or persistence. Nevertheless, the findings confirm the survival of metallic residues in SFT lesions in heat-exposed bone and demonstrate the potential of SEM-EDS for medicolegal investigations. Future research should incorporate paired analyses to provide a more comprehensive understanding of metallic trace behavior.

5. Conclusion

The present study provides an approach to addressing the challenges associated with analyzing metallic trace transfer from sharp implements in bone subjected to thermal exposure. Although SFT results in the transfer of reduced amounts of residues, unevenly distributed along the lesion edges, this research provides the first confirmed identification of such traces in burned bone sharp lesions using SEM-EDS. Fe, Cr, and Si were transferred from the knife to the ribs and persisted after burning, demonstrating the potential of this technique to distinguish sharp tool use in forensic and bioarcheological contexts. In addition, these chemical elements provide critical forensic evidence, supporting the conclusion that thermal exposure does not fully eliminate metallic traces from SFT bone lesions.

Limitations were acknowledged, including the small sample size, the use of a single knife, the substitution of porcine ribs for human bone, and the controlled mechanical strikes and heat exposure conditions, which do not replicate real-world forensic casework. In addition, the absence of paired pre- and post-burning analyses precludes assessment of residue loss or persistence following thermal exposure.

Nevertheless, SEM-EDS analysis of burned bone sharp lesions proved to be a promising approach for medicolegal investigation. Further research incorporating a wider range of sharp implements, thermal conditions, and paired pre- and post-burning analyses, is required to validate and extend these findings, thereby strengthening their forensic applicability.

In contrast to this study, previous experimental research has often removed soft tissues and skin before inflicting SFT on bone, which does not fully reflect real-world stabbing scenarios. Therefore, these tissues should be retained in future research to better approximate forensic scenarios, as they influence impact dynamics and trace transfer.

Finally, SEM-EDS analysis has proven to be a non-destructive, valuable, and rapid technique for assisting investigations involving unidentified sharp implements or for distinguishing bone damage resulting from heat exposure from SFT. However, obtaining a specific elemental signature capable of identifying a particular implement has not yet been achieved.

Clinical trial number

Not applicable.

CRediT authorship contribution statement

Ana Filipa Cunha: Writing – original draft, Methodology, Investigation, Formal analysis. **Alexandre Quintas:** Writing – review & editing. **António Pedro Alves de Matos:** Visualization, Supervision, Methodology. **Nathalie Antunes-Ferreira:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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