Int J Legal Med (2012) 126:525–531
DOI 10.1007/s00414-011-0661-7

ORIGINAL ARTICLE

The survival of metallic residues from gunshot wounds in cremated bone: a SEM–EDX study

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Received: 25 October 2011 / Accepted: 14 December 2011 / Published online: 15 January 2012

Abstract The research and analysis of gunshot residues has a relevant role in the examination of gunshot wounds. Nevertheless, very little literature exists concerning gunshot wounds on charred material. In this study, 16 adult bovine ribs (eight still with soft tissues and eight totally skeletonized) underwent a shooting test with two types of projectiles (9 mm full metal-jacketed bullet and 9 mm unjacketed bullet). Each rib then underwent a charring process in an electric oven, reaching the stage of complete calcination at 800°C. The area of each entrance wound was analyzed before and after the carbonization process via a scanning electron microscope (SEM) equipped with an energy dispersive X-ray analyzer (EDX). In each sample, metallic residues composed of lead, barium, and antimony were found. These metallic residues were thus preserved also after exposure to the extremely high temperatures reached within the oven, especially with unjacketed bullets, although the particles seem to be more irregular in shape as a result of the heating process. In conclusion, this study proved that gunshot residues survive extremely high temperatures and can be detected via SEM/EDX even in cases of charred tissues.

Keywords Forensic anthropology · Gunshot residues · Gunshot wound · SEM–EDX · Carbonization · Burning

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Introduction

In the analysis of gunshot lesions, especially entrance wounds, the search and characterization of gunshot residues (GSR) are performed in order to solve different questions the forensic pathologists have to face, such as manner of death, firing distance, or type of weapon. Gunshot residues derive from the propellant and the primer, the bullet, and the firearm itself. When a firearm is used, these residues can be detected close to the firearm (such as the shooter's hand or the gunshot entrance wound when the shot is fired at short distance) or within the tissues if the projectile has perforated them [1–4]. Over the years, several studies have been performed on the detection of GSR on many types of materials and by various methods, in order to give an unambiguous definition of "gunshot residues" and to evaluate which procedures are the most reliable for their recognition and analysis [5, 6]. At the state of the art, only the association between lead (Pb), barium (Ba), and antimony (Sb) is considered as unique for GSR particles, while other associations (like Pb–Ba, Pb–Sb, and Sb–Ba) are considered only as characteristic GSR particles. In fact, these three elements, in the form of lead styphnate, barium nitrate, and antimony sulfide, are combined in a single application for one product: the mix in the primer cap of a cartridge casing. Moreover, GSR primer particles typically show a characteristic spherical shape: this particle morphology, when combined with the elemental composition, makes GSR quite distinct from many environmental particulates [7–9].

For what may concern the techniques used to detect gunshot residues, scanning electron microscopy coupled with energy dispersive X-ray analysis (SEM/EDX) is actually considered one of the most reliable tools because it allows one to obtain both morphological information and the elemental composition of the particles. However, most of the studies which have attempted to apply these techniques to gunshot wounds deal with cadavers in a relatively good state of preservation, whereas very little literature exists on gunshot wounds on charred material: the analysis of GSR on gunshot entrance wounds in charred samples has never been performed [10–14]. What remains of these residues in charred material? And what shape does it come in? Deep morphological alterations caused by the exposure to heat (like shrinkage and crumbling) can radically modify the external appearance of a gunshot wound on bone and make it hardly recognizable. In this context, recognizing the presence of gunshot residues may be crucial in the diagnosis of a gunshot wound [15–19].

The aim of this study is to verify the potential of the scanning electron microscope in detecting gunshot residues in charred remains. No such information exists in literature in this sense, apart from a radiological experiment on cremated bone performed in parallel with the present study [20]. Furthermore, by using full jacketed bullets and unjacketed bullets, this study aims at assessing whether metallic residues like copper (Cu) and zinc (Zn), main constituents of the bullet's jacket, are detectable even after the charring process; this may be of some help for diagnosing the type of bullet.

Materials and methods

Sixteen adult bovine ribs, 20 to 27 cm in length and 2–5 cm thick, were used for this study: eight were still covered by soft tissues (principally muscle) and were named "dressed" ("D"), whereas the other eight ribs underwent maceration in boiling water and were completely skeletonized. These ribs were called "naked" ("N"). Materials are similar to those used in the parallel radiological study [20].

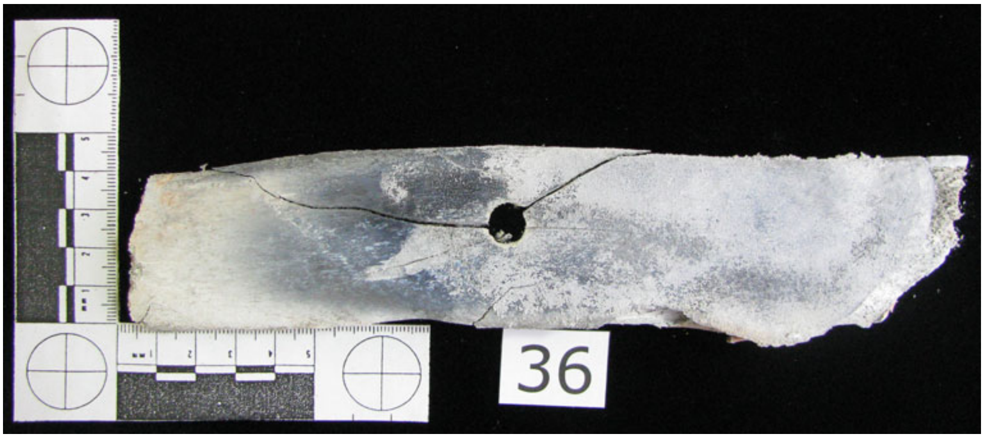
Each rib was shot at a firing ground, and the weapon chosen was a "Beretta type 98 FS" (series 92) caliber 9 mm. Two kinds of projectile were used, namely "Magtech-cbc" LRN projectiles (with unjacketed lead bullets) and "Fiocchi" 123 projectiles (with full metal-jacketed bullets).

All samples were fired with a single projectile, at near-contact range; the gunshot wound was clearly visible in all the specimens. Combining the two types of ribs and the two types of projectiles, four groups were obtained, each group composed of four samples: (1) from n°1 to n°4: NF ("naked" ribs, full metal-jacketed bullet), (2) NL ("naked" ribs, lead unjacketed bullet), (3) DF ("dressed" ribs, full metal-jacketed bullet), and (4) DL ("dressed" ribs, lead unjacketed bullet). Each sample was placed in a plastic bag and stored in a freezer before and after the trials.

In order to simulate the carbonization of human bodies, the samples underwent a "charring cycle" in an electric oven. The complete cycle lasted 24 h; during the first 12 h, the samples were heated at 800°C, whereas in the latter 12 h, the samples were left to cool. Every sample reached the morphological features of "calcined bone" (Fig. 1) with complete loss of soft tissues. The oven used to heat samples was an electric industrial machine "Vega s.r.l.", model "Vega 150", with a capacity of 150 l (maximum temperature of 1,280°C, with a rating of 10.0 kW and a tension of 230–400 V). Previous experiments performed with bovine ribs showed as benchmark temperatures for the bone's carbonization and calcination respectively 400°C ("black bone") and 800°C ("white bone"). The heating cycle was performed on eight ribs (namely ribs NF n°3 and 4, NL n°7 and 8, DC n°11 and 12, and DL n°15 and 16). In each sample, the area of the entrance wound (which includes the circular lesion and a perimetral concentric area of 3 cm) underwent SEM–EDX analysis.

Overall, for each category made of four ribs, two ribs were analyzed before and two ribs after the carbonization cycle. For what concerns the four fresh ribs still covered by soft tissues (two shot with the full metal-jacketed bullet and the other two shot with the unjacketed bullet), the analysis by SEM/EDX was not performed directly on the flesh (this SEM did not allow it) but on a thin layer of sticky inert latex

Fig. 1 Morphological characteristics of a "dressed" rib (still covered by soft tissues) after the charring cycle



previously placed and pressed onto the entrance wound so as to stick onto the largest possible number of residues. Two ribs were used as control samples; both completely skeletonized and not subjected to the shooting test. These ribs underwent investigation by SEM/EDX before and after the charring cycle. In each sample, an average of almost five residues were analyzed, although a higher number of analyses was performed on fresh samples in comparison to the charred ones, and in the "F" samples rather than in "L" samples; in the first case in fact, the analysis aimed purely at finding the typical metallic residues associated with the shot, whereas in the control sample, these residues were obviously not found; in charred samples, instead, the search was more detailed and aimed at assessing the degree of survival of the residues. In the second case, the search for metals deriving from the jacket of the projectile (copper and zinc) necessarily led us to consider a larger number of residues.

Results

First of all, metallic residues like Pb, Ba, and Sb (metals considered unique for gunshot residues) were found only in the samples hit by the bullet and not in the control samples.

As shown in Table 1, the number of residues (likely to be GSR, given their composition of Pb, Ba, and Sb) found on the entrance wound in the charred ribs was always smaller than in the fresh samples; however, a certain number of residues could always be appreciated in all the charred samples, especially when hit by unjacketed bullets. The search for metallic residues in the gunshot entrance wound led to the identification of lead as the most commonly encountered metal, found in every sample; antimony was nearly always recognized (in 15 samples of 16), both as individual particles and in association with lead or barium; in detail, barium was less frequently found (in 10 samples of 16), again as single particles or in association with the other two metals. In particular, while Pb and Sb were found in both "F" and "L" ribs, barium was more frequently found in the samples hit by the full metal-jacketed bullet, even if the total number of residues found was always greater in the samples shot by the unjacketed bullet. Another difference between the samples based on the type of projectile concerns the disposition of the residues: unjacketed projectiles seem to leave only sporadic residues on edges of the entrance wound (Fig. 2), mostly isolated and not grouped in clusters of large dimensions (these features were observed both in fresh and burnt samples, even if the burning process frequently led to a deformation of the residues, which gave them quite irregular

Table 1 Number and type of residues found in the samples

Sample			QT[a]	Metals									
				Pb	Sb	Ba	Pb+Sb	Sb+Ba	Pb+Ba	Pb+Ba+Sb	Cu	Zn	Cu+Zn
NF	1	Fresh	+ +	X		X							
	2		+ +	X	X						X	X	
	3	Charred	+	X		X							X
	4		+	X	X	X		X			X	X	
NL	5	Fresh	+ + +	X			X			X			
	6		+ + +	X		X	X						
	7	Charred	+ +	X	X		X			X			
	8		+ +	X			X						
DF	9	Fresh	+ +	X			X				X		
	10		+ +	X			X		X				X
	11	Charred	+	X					X				
	12		+	X	X	X	X				X		
DL	13	Fresh	+ + +	X			X						
	14		+ + +							X			
	15	Charred	+	X			X			X			
	16		+	X			X						

Presence of the metal/s is indicated by "X"

[a] Overall quantity of residues found in each sample

QT quantity, *NF* naked rib shot by the full metal-jacketed bullet, *DF* dressed rib shot by the full metal-jacketed bullet, *NL* naked rib shot by the unjacketed lead bullet, *DL* dressed rib shot by the unjacketed lead bullet, + = from 1 to 10 residues, + + = from 11 to 20 residues, + + + = over 21 residues

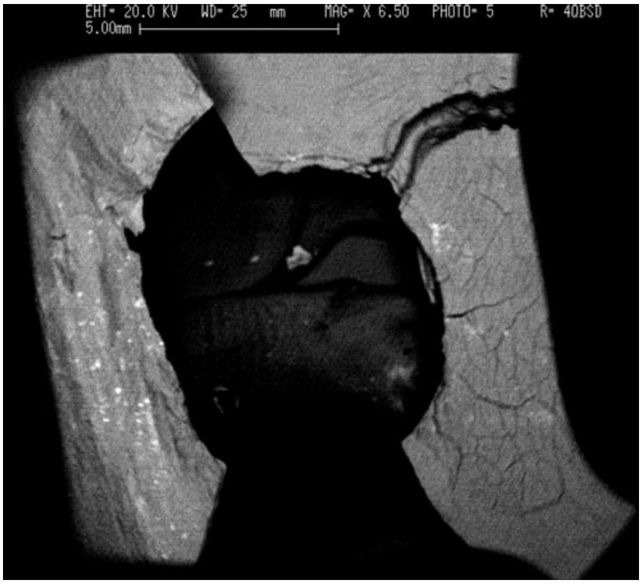


Fig. 2 Detail of an entrance wound's area in "F" charred sample

shapes). The morphological characteristics of the entrance wound edges were much different in the case of unjacketed bullets: many residues were found throughout the area, with both large and small dimensions, which consisted mainly in particles of lead and antimony or an association of these two metals (Fig. 3). Table 2 shows the results concerning shapes and dimensions of the residues: in fresh samples, the small particles are usually quite spherical (Fig. 4), while the larger agglomerates are usually of irregular shape; in charred samples, instead, residues are mainly irregular, confluent with each other, "coating" the surface of the samples (Figs. 5 and 6). In charred samples, this "smearing" of the residues (mostly Pb) was consistently observed around the edges of the entrance wound, which is enclosed in the microscopic images by a "cloud" of residues. These features ("smearing") were observed frequently also in further areas around the entrance hole.

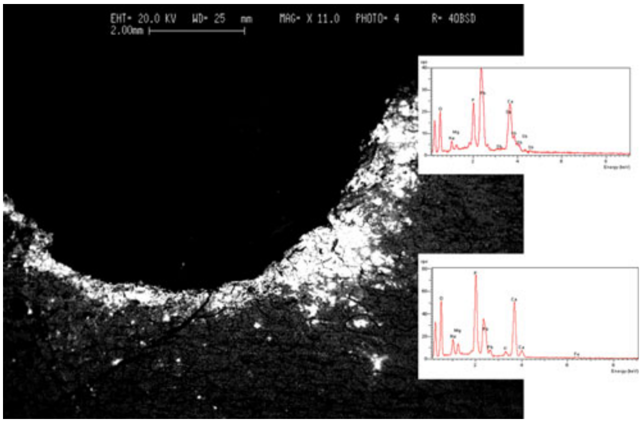


Fig. 3 Details of the edges around the entrance wound in "L" charred sample and EDX spectra particles

In "F" ribs (shot by the full metal-jacketed bullet), Cu and Zn were found in six of the eight samples (among which three were charred) both in fresh and in charred samples, as isolated residues or bigger aggregates; more irregular and indented in shape in the charred samples (Fig. 7). These residues were found both on the edges of the entrance wound, in the close areas, and even quite distant from the hole.

Finally, if there was a considerable difference in the total number of gunshot residues between ribs covered by soft tissues and skeletonized ribs, with a significantly larger quantity in the former, this difference was not observed in the samples after the charring cycle, in which the difference in the number of GSR was linked to the type of bullet and not to the presence or absence of the soft tissues before the carbonization process.

Discussion

When the forensic pathologist approaches the analysis of burnt or charred remains, several difficulties arise. Tissues and lesions within them usually may no longer be recognizable because of the effects of heat, and the differences between ante- and post-mortem lesions (due to the high temperature) may be almost impossible to detect. In this context, the macroscopic aspect of bone may be inadequate to provide a diagnosis, and the help of more advanced technologies is crucial. Several studies have focused in the last years on the differential study of lesions produced before and after the burning processes, in order to establish distinguishing criteria [21–25]; Dolinak et al. [26] specifically analyzed gunshot wounds on charred bodies by microscopic analysis of gunpowder and demonstrated the persistence of these residues. Nevertheless, no systematic studies of gunshot wounds on cremated bones have yet been performed.

In the present study, emphasis was placed on the search of GSR with SEM/EDX, one of the most reliable methods for the analysis of such residues. Many studies have already been performed on the detection of GSR by electronic microscopy [8, 10–12, 14, 27–30], confirming the high sensitivity of this method, but a study concerning the search of gunshot residues by SEM/EDX has never been performed on cremated bone.

This study confirms the validity of the electron microscope coupled with EDX in the detection and characterization of gunshot residues, confirming previous studies concerning gunshot wounds on well-preserved material, and opens up new and interesting perspectives for the study of burnt materials. Before discussing the results, it

Table 2 Classification of size and shape of the residues found by SEM/EDX in the different groups of samples (fresh and charred, jacketed and unjacketed, with and without soft tissues)

Samples	Metals											
			Pb	Ba	Sb	Pb+Sb	Sb+Ba	Pb+Ba	Pb+Ba+Sb	Cu	Zn	Cu+Zn
NF+DF fresh	Number of analyzed residues		9	2	2	2	0	1	0	3	1	1
	Shape[a]	Reg.	6	1	2	–	–	–	–	3	1	1
		Irreg.	3	1	–	2	–	1	–	–	–	1
	Diameters[b]	Min	2.5 μm	5 μm	2 μm	25 μm	–		–	1.5 μm		
		Max	45 μm	7 μm	2.5 μm	27 μm	–		–	3 μm		
		Average	17.2 μm	6 μm	2.25 μm	26 μm	–	25 μm	–	2.25 μm	2 μm	23 μm
NL+DL fresh	Number of analyzed residues		6	1	0	6	0	0	3	0	0	0
	Shape[a]	Reg.	5	1	–	2	–	–	–	–	–	–
		Irreg.	1	–	–	4	–	–	3	–	–	–
	Diameters[b]	Min	3 μm		–	12 μm	–	–	65 μm	–	–	–
		Max	55 μm		–	72 μm	–	–	120 μm	–	–	–
		Average	21 μm	4.5 μm	–	43.3 μm	–	–	85 μm	–	–	–
NF+DF charred	Number of analyzed residues		10	8	1	2	1	1	0	3	1	2
	Shape[a]	Reg.	3	3	–	–	–	–	–	1	1	–
		Irreg.	7	5	1	2	1	1	–	2	–	2
	Diameters[b]	Min	7 μm	5.5 μm		8.7 μm			–	2 μm		6.9 μm
		Max	40 μm	20 μm		15.5 μm			–	4.5 μm		21.9 μm
		Average	26.3 μm	12.9 μm	6.4 μm	12.1 μm	16.5 μm	82 μm	–	3.2 μm	4.4 μm	14.4 μm
NL+DL charred	Number of analyzed residues		7	0	2	12	0	0	2	0	0	0
	Shape[a]	Reg.	2	–	–	3	–	–	–	–	–	–
		Irreg.	5	–	2	9	–	–	2	–	–	–
	Diameters[b]	Min	11.5 μm	–	3.5 μm	9.5 μm	–	–	45 μm	–	–	–
		Max	46 μm	–	6.8 μm	69 μm	–	–	65 μm	–	–	–
		Average	28.8 μm	–	5.15 μm	38.8 μm	–	–	55 μm	–	–	–

Reg. regular, *Irreg.* irregular, *Min* minimum, *Max* maximum, *NF* naked rib shot by the full metal-jacketed bullet, *NL* naked rib shot by the unjacketed lead bullet, *DF* dressed rib shot by the full metal-jacketed bullet, *DL* dressed rib shot by the unjacketed lead bullet, *DL* dressed rib shot by the unjacketed lead bullet

[a] Number of residues with regular/irregular shape

[b] In cases where the residue was irregular in shape, the longest diameter was taken

is necessary to clarify that for logistical reasons, in each sample, not all the observed residues were analyzed, but only a fraction of these (about six for each sample, as already stated), thereby leading to a purely qualitative evaluation, without performing a quantitative analysis for which further and more detailed studies are needed.

First of all, the charring process seems to lead to an overall decrease in the number of gunshot residues found in the area of the entrance wound: this is probably due to the volatilization of the main constituents of GSR (Pb, Ba, and Sb), present mainly as oxides in the propulsive charges. However, the electron microscope does not fail in detecting metallic residues. In fact, in each charred sample, residues of Pb, Ba, and Sb were found, although fewer in number than in the case of fresh unburnt samples. However, this permits one to verify the surviving gunshot residues in the analyzed zone, thus confirming the shot or the passage of the projectile through the area even when it cannot be morphologically identified as a gunshot wound anymore; therefore, this information confirms that gunshot residues are able to survive even at high temperatures (800°C reached within the oven). This aspect is even more evident in the samples hit by the unjacketed bullet (where a greater number of residues were observed both in fresh and charred samples): probably the lack of the jacket around the bullet allows a greater release of residues when the projectile passes through the sample; the jacket, however, may be an obstacle to the release of residues, and in the samples shot by the full metal-jacketed bullet, the number of residues is lower. As a sign of the presence of GSR in every charred sample analyzed, the three main constituents of GSR (Pb, Ba, and Sb) were found in each one, especially lead which showed the highest concentrations. Furthermore, interestingly, the two main constituents of the bullet's brass jacket, namely Cu and Zn, were observed in the "F" samples (shot with the full metal-jacketed bullet), both fresh and charred. The present

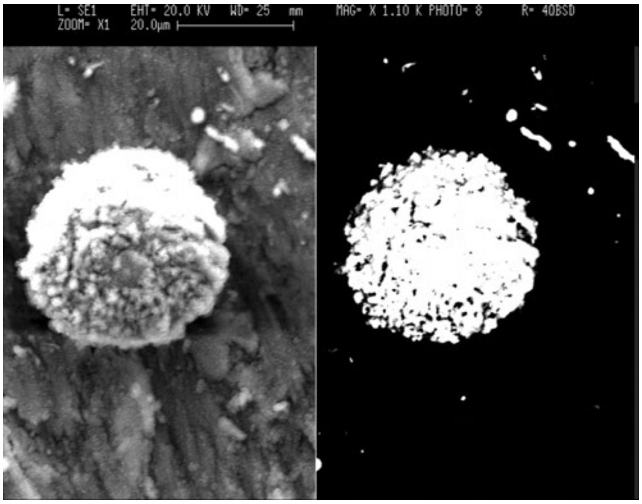


Fig. 4 Particle of lead in fresh sample

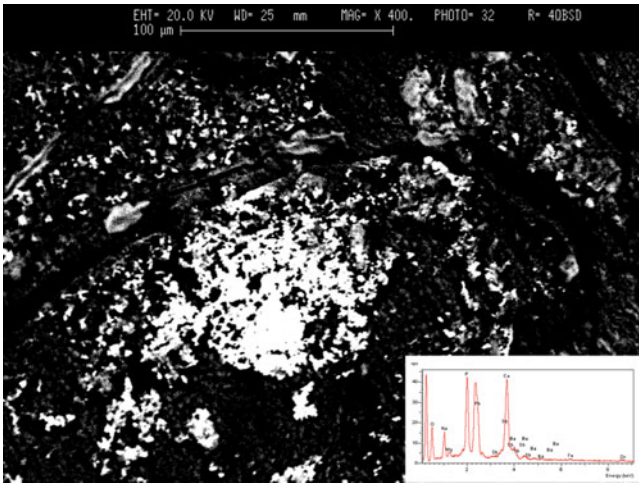


Fig. 6 Spread residues in charred sample: EDX analysis confirmed presence of Pb, Ba, and Sb

results therefore, if confirmed by further studies on a larger population, may be the first step for proving not only the passage of the projectile through the sample even after the carbonization, but also the type of projectile (jacketed or unjacketed). However, further studies are needed in order to evaluate the different types of bullets and a higher number of samples.

A specific discussion concerns the shape and the position of the metallic residues found; after the carbonization process, in fact, residues seemed to lose the typical characteristics of GSR: they no longer had a round shape and small dimensions, but tended to cluster, to "spread" and to take on a jagged, more irregular shape. As previously shown, residues with the exposition to high temperatures took on a more notched, indented, and sometimes unusual shape, due also to the confluence of the neighboring residues. This could be a very important aspect that should be taken into serious consideration for the analysis of carbonized residues. Concerning the position of GSR, the number of residues on the edges of the entrance wound was higher when the gunshot wound was made by an unjacketed bullet (especially in the burnt samples, where residues are spread around the area of the entrance and their shape is quite clearly defined); this information may be useful for a more precise localization of the entrance wound. Therefore, when the gunshot wound is not macroscopically recognizable as such, the detection of GSR with SEM/EDX can prove to be a useful tool provided contamination can be ruled out. In conclusion, this study demonstrates the persistence of gunshot residues on bone even after the

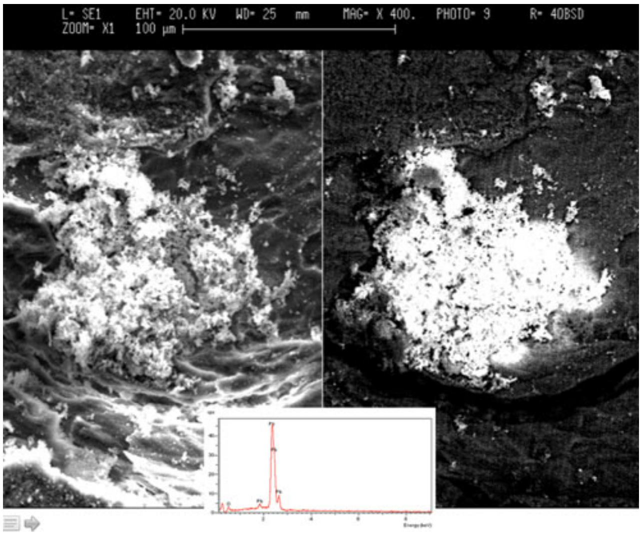


Fig. 5 Irregular particle of lead in charred sample

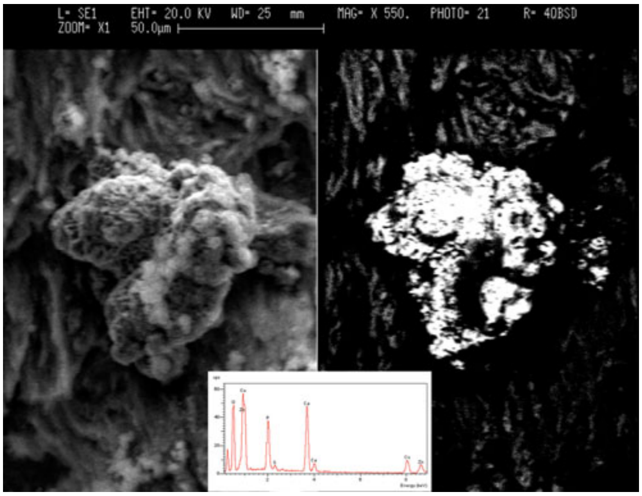


Fig. 7 Irregular agglomerate of Zn and Cu in charred sample

exposure to high temperatures; however, further studies must be performed, which should analyze a higher number of samples.

Acknowledgments We thank Mr. Giancarlo Scarpa and the staff of the firing ground of Pavia for their collaboration during the experiment and the butchery of Parma for the bovine ribs.

Ethics The study was performed according to the local ethics committee.

Conflict of interest The authors declare no conflict of interest.

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