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TECHNICAL NOTE

ANTHROPOLOGY

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Gunshot Residues on Dry Bone After Decomposition—A Pilot Study

ABSTRACT: Very little literature exists concerning radiochemical and microscopic analyses of gunshot wounds in decomposed material, and even less concerning skeletonized samples; the most advanced technologies may provide useful indications for the diagnosis of suspect lesions, especially if gunshot wounds are no longer recognizable. However, we know very little of the survival of gunshot residues (GSR) in skeletonized samples. This study examined nine gunshot wounds produced on pig heads which then underwent skeletonization for 4 years, and four gunshot entries on human heads from judicial cases which were then macerated to the bone in water; the samples underwent scanning electron microscopy coupled with energy dispersive X-ray (SEM-EDX) analysis. Positive results for GSR were observed only in four of the nine animal samples and in all four human samples. Among the human samples, two lesions showed Pb and Sb, one lesion only Pb, and one Pb, Sb, and Ba. This pilot study showed the survival of GSR in skeletal material and therefore the crucial importance of SEM-EDX analyses on skeletonized material. Further studies are needed in order to ascertain the role of environmental modifications of GSR.

KEYWORDS: forensic science, forensic anthropology, gunshot wounds, gunshot residues (GSR), SEM-EDX, bone tissue

Analysis of gunshot residues (GSR) on gunshot lesions is of the utmost importance in forensic pathology and anthropology, as shown by the large amout of research on this topic. GSR is generally made of particles released by the primer, the propellant, and the bullet itself. This explains the large variability of residues released by the gunshot; however, at the moment, metals considered as specific for GSR are lead (Pb), antimony (Sb), and barium (Ba), which are included in the propellant and the primer (1,2): finding all three metals within a sample is strong evidence for a gunshot lesion according to literature. In detail, particles of Pb, Ba, and Sb are considered "unique" for the diagnosis of gunshot wounds according to the classification by Wolten et al. (3,4).

Unfortunately very little is known concerning the survival of GSR in putrefied material and even less in skeletonized samples. The diagnosis of gunshot wounds in cases of advanced decomposition and skeletonization is often difficult to sustain, and it may be necessary to verify if a lesion is indeed a gunshot wound in the case of fragmentary material or of unusual morphology of the lesion. Thus verifying the feasibility of GSR detection on decomposed material is crucial. At the moment, few experimental studies exist concerning the survival of GSR in decomposed material; a recent study verified the persistence of Sb detected by Neutron Activation Analysis (NAA) up to 16 weeks on putrefied skin, also in cases of buried samples (5). Berryman et al. (6) noted that GSR were detectable in case of defleshed samples, even after removal of the periostium layer. These scarce data seem to suggest that GSR may survive decomposition modifications of the bone surface.

Actually, several methods exist for the detection of GSR. NAA, atomic absorption spectroscopy (7), inductively coupled plasma mass spectroscopy (8), millimeter-X-ray fluorescence analysis (9), and scanning electron microscopy coupled with energy dispersive X-ray (SEM-EDX) analysis are the most commonly used investigative methods. This last technique combines morphological information with the chemical analysis of each particle detected (10,11). It is widely available, relatively cheap, and the search procedure can be automated (12,13). To the best of our knowledge, most of the articles on the detection of GSR by SEM/EDX have so far examined GSR collected from the hands of the persons involved in shooting and from other objects near a gun after firing (10,11,14–16)—very few articles on GSR from gunshot in bone have been published. The previously cited study from Berryman et al. (6) concerns detection of GSR by SEM and was conducted on pork ribs which underwent firing tests and decomposition in a plastic bag for about 5 days. This study showed the sensitivity of the SEM technique in detecting GSR on bone (6). Other authors tested for GSR in human bone samples with a spectroscopic technique by means of the proton-induced emission (PIXE) analysis system to verify the presence of Pb in the bone of a murder victim (17,18). In this study the authors presented three cases in which the use of PIXE was decisive for confirmation of the presence of Pb around gunshot wounds on the bone when soft tissues were no longer available. Although PIXE allows one to discriminate particles of GSR which are difficult to distinguish with SEM-EDX, this technique is not routinely used as a primary source of GSR because it has very high costs (12).

SEM-EDX indeed seems to be more user-friendly and cheap. For these reasons it is currently one of the most advanced

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technologies applied to the analysis of lesions for the detection of residues, not only in cases of gunshot wounds, but also on other types of lesions such as cut marks: in these cases SEM-EDX technology can provide relevant information concerning the chemical characteristics of the blade (19–23).

The present study aims at carrying out a pilot SEM-EDX analysis of GSR on (i) skeletonized gunshot wounds in pig bones and (ii) macerated gunshot wounds on human bone, in order to verify their survival, and obtain information concerning the applicability of this technology on bone. The procedures followed are in accordance with the ethical standards of the responsible committee on human experimentation.

Material and Methods

Gunshot Residues on Skeletonized Pig Samples

Nine gunshot wounds were produced on five pig heads (mandible and forehead) during a shooting test at a firing ground. All the animal samples had died from causes independent from the experiment. The chosen weapon was a Franchi revolver .38 special, with a cartridge full metal jacket .38. Shooting distance was 5 cm. Among the nine lesions, five were left to decompose in open air and four under 20 cm of soil in pots; the samples were kept indoors in order to avoid eventual modification by rain, wind, and other unpredictable climatic conditions. The samples reached complete skeletonization within the 16–20 week period following the beginning of the experimental project; after 4 years the bone lesions were recovered and tested for GSR research by SEM-EDX.

In Table 1 the information concerning the nine bone lesions and the different conditions of decomposition, are reported.

Gunshot Residues on Human Samples

Four gunshot wounds on the calvarium were collected from four shooting victims for judicial purposes. Sample number 1 was an entrance wound on the cranium produced by a 9×21 mm Beretta 98 F/S, at contact range. Sample number 2 consisted in a gunshot entrance wound on the cranium by a .22 weapon at an unknown distance. Sample number 3 consisted in a gunshot entrance wound on the cranium produced by a 9×21 mm Beretta 925B at contact range. Sample number 4 was a gunshot entrance wound on the cranium produced by a caliber 7.65 revolver at a short distance (Table 1).

The human bone samples underwent a "fake" decomposition process by maceration for 1 week to remove the residual soft tissues: in this case the samples were left in distilled water to "decompose" at room temperature.

Pig and human samples were shot at different distances and underwent two different modalities of decomposition and skeletonization because initially part of two different experimental sets. Human samples were taken for judicial purposes and cleaned with a maceration method in order to study the presence and aspect of bone lesions. Pig samples were part of a study on GSR on decomposing soft tissue (5) in soil. Given that both underwent SEM-EDX analysis, once skeletonized, we decided it would be more useful to combine the results of these different settings concerning skeletonized gunshot wounds.

Human and pig bone lesions were excised from the surrounding bone and were then metallized by carbon coating for SEM-EDX analysis. The samples were examined by a Cambridge Stereoscan 360 with electron gun, vacuum pump, and image acquisition software (EDX spectrometry with detector from 138 eV to 5.9 KeV; Oxford Link Pentafet, Oxford, UK). The search for residues was performed by the SEM operator who visually verified the presence of bright particles which were suggestive for metallic residues and then performed EDX analysis. Negative controls consisting of a piece of cranium placed in soil and a piece of cranium taken at autopsy and subsequently macerated were included in the study.

Results

The SEM-EDX results of the two different groups of samples are reported in Table 2. Positive results for GSR were observed only in four of the nine animal samples and in all four human samples. Among the human samples, all the gunshot wounds were positive for GSR (two lesions showed Pb and Sb, one lesion only Pb, and one Pb, Sb, and Ba).

Among the animal samples, two gunshot wounds left in open air were positive for Pb or Ba; among the four samples buried in soil, two gunshot wounds were positive for Pb and Ba. Sb was not observed in the animal samples. The particles were actually mixed; in some cases, they were irregular in shape (Fig. 1); in others spherical (Fig. 2). Particles were always found very close or on the edge of the bone lesion. Negative controls produced negative results for Pb, Sb, and Ba.

TABLE 1—*Details of firing conditions of the gunshot wounds on skeletonized human and animal samples.*

	Area of the Entrance Wounds	Weapon	Cartridge	Distance of Shot
Animal Samples				
1	Cranium	Franchi revolver	.38	5 cm
2	Cranium	Franchi revolver	.38	5 cm
3	Cranium	Franchi revolver	.38	5 cm
4	Cranium	Franchi revolver	.38	5 cm
5	Cranium	Franchi revolver	.38	5 cm
6	Cranium	Franchi revolver	.38	5 cm
7	Cranium	Franchi revolver	.38	5 cm
8	Cranium	Franchi revolver	.38	5 cm
9	Cranium	Franchi revolver	.38	5 cm
Human sample				
1	Cranium	Beretta 98 F/S	9×21 mm	Contact
2	Cranium	Unknown	.22	Unknown
3	Cranium	Beretta 925B	9×21 mm	Contact
4	Cranium	7.65 revolver	7.65	Short distance

TABLE 2—*Results of scanning electron microscopy coupled with energy dispersive X-ray test on animal samples.*

Pig Samples	Enviroment of Decomposition	Gunshot Residues Pb, Ba, Sb
1	Open air	–
2	Open air	–
3	Open air	Pb
4	Open air	–
5	Open air	Ba
6	Buried in soil	–
7	Buried in soil	Pb, Ba
8	Buried in soil	Pb, Ba
9	Buried in soil	–
Human Samples		Gunshot Residues Pb, Ba, Sb
1		Pb, Sb
2		Pb, Sb, Ba
3		Pb, Sb
4		Pb

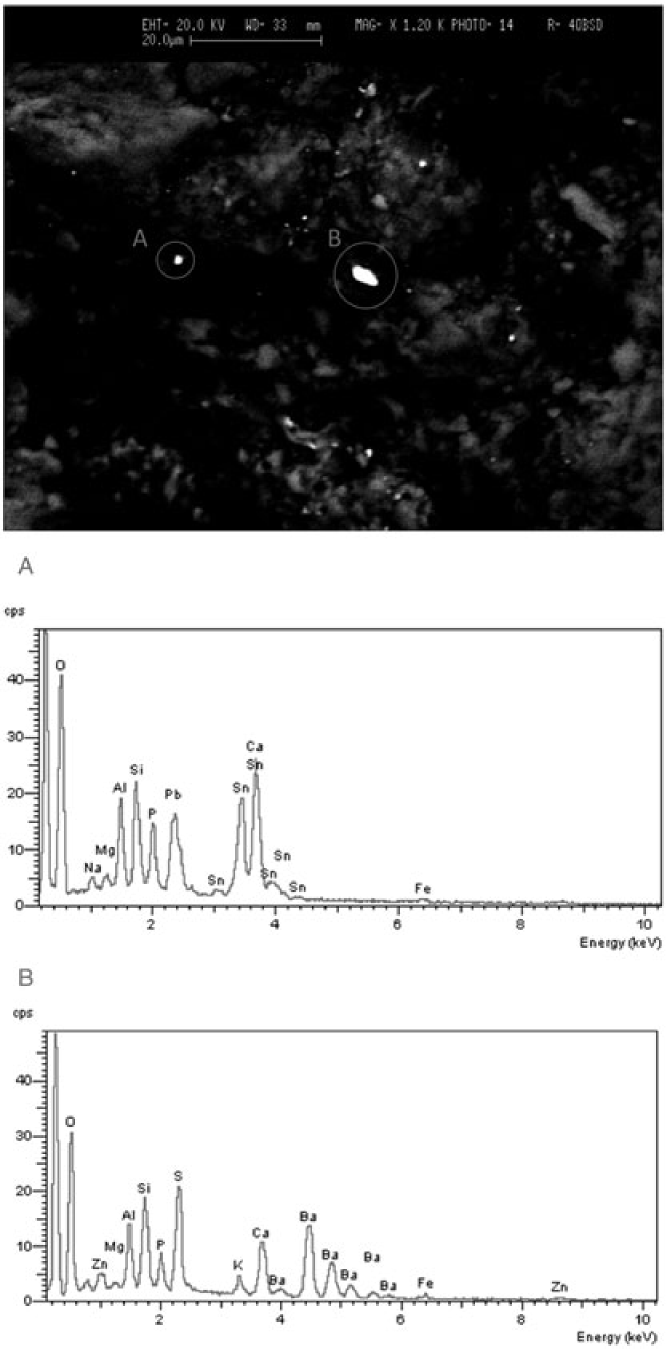

FIG. 1—*SEM image and EDX spectrum of the particles found on the edge of the wound on pig sample eight left to decomposed under 20 cm of soil. The wound was produced by a .38 special Franchi revolver at a distance of 5 cm. The EDX identified Pb on the left (A) and Ba on the right (B).*

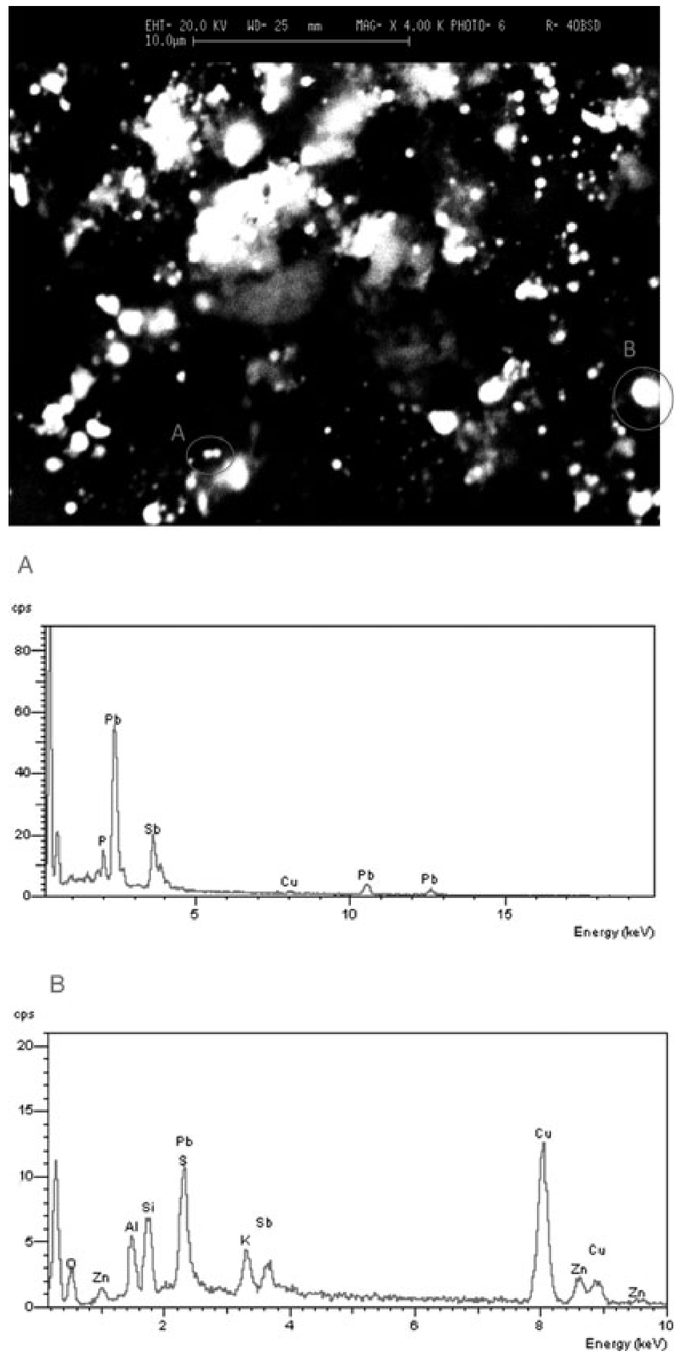

FIG. 2—*SEM images and EDX spectra of the particles found on the gunshot wound entrance of the human sample number one produced by a caliber 9×21 mm Beretta 98 F/S at contact. The EDX analyses identified residues of Pb and Sb.*

Discussion

The macroscopic identification and interpretation of gunshot wound evidence is relatively easy in the early stages of decomposition but is very difficult if not impossible in the late stages because the changes due to putrefaction or degradation can modify the morphology of the gunshot lesion (5,24,25). Thus if the typical gunshot wound fracture pattern is no longer recognizable, radiochemical tests NAA, PIXE, and in particular SEM-EDX may be of help (3,4,16).

In detail a recent study showed how in cases of putrefied soft tissue remains NAA testing could be useful to verify whether a lesion is in fact a gunshot wound (5). Moreover Berryman et al. showed that the use of SEM-EDX revealed GSR particles on pork ribs shot at a distance of 1–6 feet (6).

The present study shows that some residues survive in decomposed and macerated bone. Positive results for GSR were observed in all four human bone specimens and only in four of the nine animal bone samples. Human samples were shot with different weapons at short or contact distance; Pb and Sb were detected by SEM-EDX testing in two samples, Pb, Sb, and Ba in one sample, and only Pb in the last. The four human bone samples therefore gave interesting results for GSR, regardless of their maceration in water. This confirms the persistence of GSR in "washed out" bone.

As concerns the animal samples, only four samples were positive: two samples (which had skeletonized in open air) showed Pb and Ba, respectively, and two samples (which had been buried in soil) showed Pb and Ba. Sb was never detected. Thus some GSR may still be recognizable after 16 weeks, and their survival seems to be independent from the specific environment of decomposition, though it is certainly sporadic. This seems to justify the application of SEM-EDX to skeletonized cases which present some difficulty in the intepretation of lesion morphology.

The reason why human samples gave better results than animal samples may be that most human samples were shot at contact or at very close range, whereas the pig bones were always shot at a 5 cm distance. Furthermore, the clean macerated human samples clearly underwent less diagenetic and taphonomic processes with respect to the animal bones which were left for a much longer period in soil or at room temperature.

One question which arises is whether we are in front of bullet wipe or primer residues. Usually GSR produced by the propellant are spherical in shape (4) and can be found around the gunshot lesion, whereas the metallic residues transferred from the bullet wipe tend to be irregular in shape and placed on the edge or very close to the entrance wound. The fact that particles are mixed in shape suggests both bullet wipe and propellant; as concerns their distribution, this may have a limited significance since the movement of decomposing tissue which used to cover the lesion may have shifted particle location. Further experiments may help solve this dilemma.

The study also showed that decomposition may change GSR profile; colliquation of soft tissues may cause the spreading or loss of GSR, and this may explain the modification of the profile of GSR recorded in decomposed material, regardless of whether it decomposed in water or in soil: this also requires further studies in order to verify the exact influence of decomposition and environmental conditions on the alteration of the GSR profile.

Another relevant field of study concerns the precise correlation between the amount of residues, shooting range, and decomposition; however, this was intended as a pilot study, based on few samples, which aimed only at verifying the persistance of GSR on decomposed material: the in-depth analysis or correlation between the amount of GSR and the degree of decomposition requires further, even statistical, analysis, with consequent need for a higher number of both animal and human samples.

These results are but another small step toward the study of the survival and reliability of GSR on skeletonized material. Further studies will focus on the environmental variables which may influence preservation or loss.

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