

A Comparative Analysis for the Identification of Gunshot Residues in Burnt, Decomposed Tissue and Cremated Bone Samples

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Abstract

Forensic anthropologists, pathologists, and analysts face challenges when undertaking the identification of gunshot wounds, particularly in burnt, decomposed tissue and burned bone samples. Gunshot wounds and marks can be unrecognizable in decomposed or charred remains, posing a challenge for forensic analysts doing qualitative examinations. Burning of human remains increases the difficulty of examining samples due to alterations made by fire or other sources of intense heating or burning.¹ It is also difficult to determine the precise entry of the projectile in burnt remains. Severe alterations of tissues can make a wound macroscopically unrecognizable or create surfaces that look like but are not gunshot wounds.¹ In such cases, quantitative instrumental methods such as inductively coupled plasma-mass spectroscopy and inductively coupled plasma-optical emission spectroscopy are ideal and definitive methods of inorganic analysis for trace metals in gunshot residues (GSR) in charred and decomposed remains. In studies to differentiate between two different bullet types (jacketed and non-jacketed) on absorbed tissue samples, quantitative analysis detects specific inorganic metal residues left by gunshot wounds—such as antimony, barium, lead, copper, and iron—in burnt/cremated bone and decomposed tissue samples. The quantitative instrumental methods tested here enable forensic researchers to detect GSR despite excess heat, charring, and decomposition processes in harsh environments, validating an important tool for detection of inorganic residues after degradation, by burning or decomposition, of evidence of gun-related violence and crimes.

Introduction

Gun-related violence and crime rates have risen steadily in the United States, and precise identification and analysis of gunshot residues (GSR) are needed to bring criminals to justice. Many gun-related crimes result in either the death of individuals or their compromise by severe internal injuries. General identification of GSR is determined using a gross injury examination, a qualitative analysis, at an autopsy. The interpretation of gunshot wounds can be difficult even when the deceased individual is well preserved, because the characteristics of gunshot wounds vary greatly based on the type of firearm

and ammunition used, the range of fire, and the location of the wound on the body.¹ Postmortem factors such as decomposition, burial, and insect activity make the identification of gunshot wounds even more challenging. Usually, forensic medical examiners and pathologists cut tissue sections where GSR is located and perform histological procedures to determine the presence of gunshot residues; however, microscopic examination may be useless if tissue and bone samples are burned, cremated, or decomposed. Hence, the ability to chemically detect and identify GSR around a suspected gunshot wound is desirable. Therefore, a precise method of identification is required for quantitative analysis in decomposed tissue samples. Inductively coupled plasma-mass spectroscopy (ICP-MS) can be utilized to identify and quantify GSR from two different bullet types, jacketed and unjacketed.

Forensic anthropologists face similar challenges when undertaking the identification of gunshot wounds in tissue and bone samples subjected to fire. Burning or carbonization of human tissue makes it difficult for forensic anthropologists to identify the specific lesions made by the projectile(s) in bone. Severe alterations of the tissues can make a wound macroscopically unrecognizable or create surfaces that resemble gunshot wounds but are not.¹ A chemical or instrumental analysis not only can help forensic anthropologists and pathologists, but will precisely discern the composition and quantity of GSR fragments in areas where entrance wounds are expected to be located. Thus, a chemical analysis will improve the analysis and yield optimum results for GSR, in contrast to a qualitative examination of the remains in which error may readily occur.

Various techniques exist for quantifying and identifying metal and primer composition in GSR. However, a quantitative chemical analysis of GSR has rarely been conducted on burnt bone and tissue samples. A precise identification and quantification of GSR and components in degraded tissue can be definitively determined utilizing inductively coupled plasma–optical emission spectroscopy (ICP-OES) or inductively coupled plasma–mass spectroscopy (ICP-MS). Two studies were performed to quantify heavy metal residues in GSR in decomposed tissue and in burnt tissue. ICP-MS and ICP-OES were the instrumental methods used to analyze trace inorganic metals in these tissue samples.

Composition of GSR

GSR, which may also be known as cartridge discharge residue, is comprised of particles produced during the discharge of a firearm.² When a cartridge round is fired from a firearm, combustion products from the primer and the propellant are released simultaneously at great speeds. GSR is composed of unburned and partially burnt propellant powder, particles from the ammunition primer, smoke, grease, lubricants, and metals from the cartridge as well as from the weapon itself.³ Organic compounds originate from propellant and firearm lubricants, taking the form of partially burned gunpowder particles. Inorganic residues consist of inorganic metals such as lead (Pb), barium (Ba), and antimony (Sb), and represent the primer material at the base of the bullet. These combustion materials from the primer, propellant, and other sources escape from weapon openings and vaporized materials that solidify into particulates.² Less common inorganic elements in bullets include aluminum (Al), sulfur (S), tin (Sn), calcium (Ca), potassium (K), chlorine (Cl), copper (Cu), strontium (Sr), zinc (Zn), titanium (Ti),

and silicon (Si). These metals may be incorporated in bullet casings, bullet core, and possibly other sources of primers like mercury fulminate.⁵ Organic GSR primarily comes from materials derived from the propellant powder and includes compounds classified either as low explosives or additives based on their chemical composition. Low explosive material may consist of nitrates, nitrites, nitroglycerin, potassium chlorate, smokeless powder, black powder, and many other compounds required to undergo ignition by the primer to achieve the outward expansion of the bullet. The organic and inorganic material can be fit into a small cylindrical cartridge as shown in Figure 1.

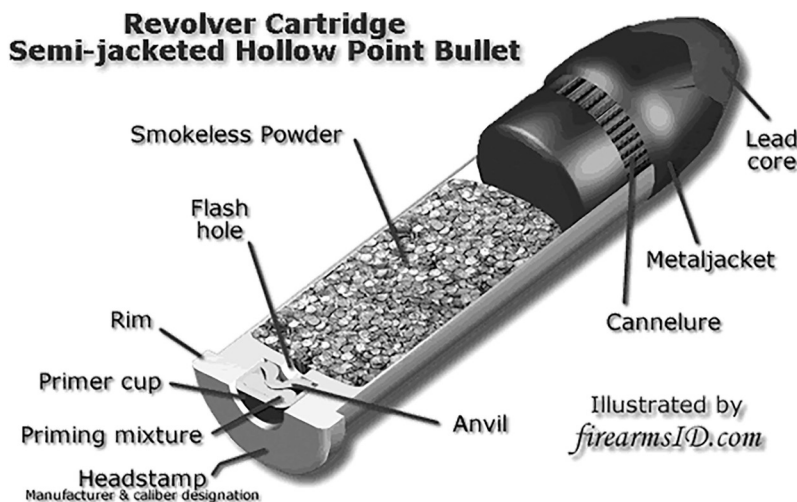


Figure 1. Revolver cartridge, semi-jacketed hollow point bullet.⁴

The cartridge case, bullet, bullet coating, and metal jacket shown in Figure 1 contain specific elements that can be detected via instrumental analysis. Virtually all cartridge cases are made of brass (70% copper, 30% zinc). A few cartridge cases have a nickel coating. Primer cases are of similar composition to that of cartridge casings; they are made of brass (Cu, Zn). Bullet cores are most often lead (Pb) and antimony (Sb), with a very few bullet cores having a ferrous alloy component to it. Bullet jackets are usually brass (90% copper with 10% zinc), but some are a ferrous alloy and some are aluminum.⁴ Some bullet coatings may also contain nickel, since bullet production is inexpensive despite environmental hazards. Understanding the components of bullet cartridges and GSR, forensic scientists can perform various instrumental analyses for the precise quantitative detection of heavy metal inorganic- and organic-based explosive residues found in gunpowder.

ICP-MS Analysis of Decomposing Tissue Samples

Replicating a previously reported study by Udey, Hunter, and Waddell Smith,⁵ a study was performed on three euthanized pigs to generate the quantitated data in Tables 2 and 3. Two dead pigs were shot 12 times each using a Smith & Wesson .357 Magnum

revolver.⁵The first pig carcass was shot with a 158 grain copper-jacketed hollow point ammunition with a jacketed bullet base. The second was shot with the 158 grain non-jacketed lead ammunition.⁵The supplier for both ammunitions was the Remington Arms Co. Inc. The gun barrel was cleaned after each shot, and the chamber was cleaned before reloading to prevent possible cross-contamination that could arise from this analysis. All three pig carcasses were then transported to the research field, where the control pig was stabbed 12 times to create open wounds to attract insect activity similar to the shot pigs. The pig carcasses were placed inside separate wire cages to protect them from predators while still allowing exposure to the environment.⁵ The wounds and histological samples were collected over a period of 49 days. One tissue section was removed from each tissue sample from the two gunshot pigs and the control carcass, and was then run through microwave digestion and ICP-MS to quantitate and analyze the elements of interest.

The researchers compared the decomposed tissue and a fresh tissue sample. It is expected that a decrease in concentration for various GSRs will occur via decomposition. The five elements of interest in this study are: Iron (Fe), Copper (Cu), Antimony (Sb), Barium (Ba), and Lead (Pb). They were quantitated using ICP-MS method, and the limits of quantitation were calculated and reported for each metal species. The limit of quantification (LOQ) in Table 1 describes the smallest concentration of an analyte that can be reliably measured by an analytical procedure or instrumental method, in this case ICP-MS. All elemental signals were monitored in both the undiluted and diluted tissue sample sets. Iron (Fe) and copper (Cu) are present in relatively low levels throughout the study. These elements were quantitated using the undiluted digest samples so that signals remained within the linear range of the calibration curve. The elements antimony (Sb), barium (Ba) and lead (Pb) were more concentrated and quantitated in the diluted samples to ensure that sample signals were within the linear calibration curve range and did not saturate the detector.⁵For all elements under analysis, errors were less than ten percent (<10%), as shown in Tables 1 and 2, indicating that the instrument was accurately quantitating the elements in all samples and that the instrumental technique was a highly sensitive method for analyte detection.

Table 1. Limit of quantification (LOQ) values for fresh and decomposed tissue samples.⁵

Element	LOQ (µg/L)	
	Fresh Tissue Study	Decomposition Study
⁵⁶ Fe	5	5
⁶³ Cu	5	5
⁶⁶ Zn	10	10
¹²¹ Sb	<0.1	<0.1
¹³⁸ Ba	0.5	<0.1
²⁰⁸ Pb	0.25	0.25

Table 2. Average reference material recovery values for fresh and decomposed tissue samples.⁵

Element	Average % Error	
	Fresh Tissue Study	Decomposition Study
⁵⁶ Fe	7	0.5
⁶³ Cu	1	6
¹²¹ Sb	2	4
¹³⁸ Ba	6	7
²⁰⁸ Pb	3	5

The analyte concentrations are calculated throughout the decomposition stages from Day 0 to Day 49; the range of mean concentrations for each element of interest is shown in Table 3. The concentrations encompass a certain degree of variability as the highest element concentrations did not necessarily occur on day 1 of the decomposition process and the lowest concentrations did not necessarily occur on day 49.⁵ The analyte concentrations in the decomposed tissue samples did not produce slight or observable changes as one would expect throughout the decomposition process. The mean element concentrations were calculated and recorded for six tissue samples from each bullet type and collected throughout the study at different stages of decomposition on days 0, 5, 14, 24, 34, and 44 in the process.⁵

Table 3: Mean element concentration ranges in decomposition process. ⁵

Element	Range in Element Concentration (µg/g) Through Moderate Decomposition		
	Control	Jacketed	Nonjacketed
⁵⁶ Fe	83–235	348–720	242–438
⁶³ Cu	12–27	306–835	99–166
¹²¹ Sb	10–15	197–3677	1722–3923
¹³⁸ Ba	ND*	1721–18159	5057–16599
²⁰⁸ Pb	0–11	702–11010	42753–131245

*ND indicates “not detected.”

As shown in Figure 2, the analytes of interest in this study show higher concentrations in tissue with gunshot wounds relative to the control tissue and procedural blank samples, indicating that this a sensitive method. Mean element concentrations for copper and barium at the 99% confidence level and for iron and lead at the 95% confidence level were significantly higher in tissues shot with full-jacketed

ammunition compared with the control. For tissue samples shot with non-jacketed ammunition, all elements were present at significantly higher concentrations in the shot tissue compared with the control tissue at the 99% confidence level.⁵ All elements in this study are suitable for differentiating tissues with gunshot wounds from control tissues throughout moderate decomposition processes.

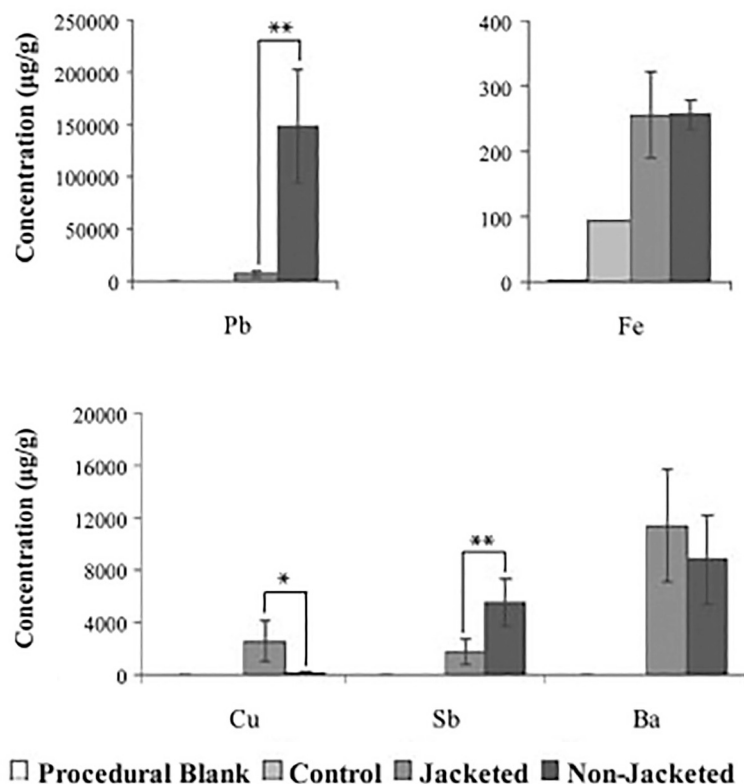


Figure 2: Mean concentrations for different element/analyte species under study via ICP-MS.⁵

The variation in elemental concentrations between tissue samples from carcasses shot with jacketed and those shot with non-jacketed bullet types provides evidence of the bullet make and composition, and differentiates the two bullet types. Higher concentration levels of lead (Pb) are detected in non-jacketed bullet ammunition while copper (Cu) is detected at higher concentrations in jacketed bullet ammunition.

The results of this decomposition study for the comparison of two bullet types (jacketed and non-jacketed) in decomposed tissue samples suggest that these specific metal species can still be detected via ICP-MS when conditions do not favor qualitative analyses. Despite the tissues' having undergone a 49-day period of decomposition, the five metal species were still detected with a highly sensitive instrument coupled method (ICP-MS). It was thought that certain metal species would dissipate in the environment

throughout the 49-day decomposition period due to temperature and weather effects. Other factors such as insect activity, decomposers, and soil acidity could very well affect the detection of these analytes. This study provided strong supporting evidence that elemental inorganic residues in GSR can still be detected and quantitated after decomposition processes. It is evident from this study that trace amounts of heavy metal GSR components can still be detected even when a murderer dumps or disposes of the body for decomposition.

ICP-OES Analysis of Cremated Bone and Burnt Tissue Samples

As published in 2013, forensic researchers at the University of Milan analyzed GSR in cremated bone and bone fragments from both full-metal-jacketed projectiles and non-jacketed projectiles,¹ and the present study replicated their inquiry. The morphological features of a gunshot wound on bone depend on many variables; these include firing distance, type of ammunition, type of firearm, and location of the gunshot wound on the body.¹ The typical morphology of a gunshot wound, however, can vary greatly when it undergoes severe alterations by charring or cremation. These types of alterations make it extremely difficult for forensic anthropologists and forensic pathologists to examine the morphological striations and markings caused by projectile firing on bone. A chemical analysis is the only viable and definitive option for trace analysis of GSR components, particularly for residues of heavy metals commonly found in bullets. Little research has been performed on burnt and cremated bone samples. This quantitative analysis involves inductively coupled plasma-optical emission spectrometry (ICP-OES), which works in conjunction with a qualitative analysis or approach for comparison using a scanning electron microscope coupled with a dispersive x-ray analyzer (SEM-EDX). Although the SEM-EDX method of analysis does allow for identification and detection of metal residues, quantification is deemed a subjective analysis. It was decided to perform this study with ICP-OES to verify whether this method yielded positive results on burnt bone, allowing for a more objective quantification of the chemical residues in GSRs.¹

This study evaluated the usefulness and reliability of the ICP-OES instrumental analysis on burned samples. This method was used to quantify trace amounts of GSR on charred tissue and bone samples. The study involved sixteen adult bovine ribs, eight of which contained soft tissues, while the other eight ribs were completely skeletonized. These samples were shot using two kinds of projectile, a 9-millimeter full metal-jacketed projectile and an unjacketed projectile. The analysis allows for the identification of GSR from both bullet types in charred tissue and bone fragments. Each rib was shot at a firing ground, with a Beretta-type 98 FS (series 92) caliber 9 mm. Two kinds of projectile were used: the Magtech-cbc LRN projectiles (with unjacketed lead bullets) and the Fiocchi 123 projectiles (with full metal-jacketed bullets).¹ The gunshot wound after discharge displayed clear visible circular lesions in all samples, along with several radiating fractures on the bone. The samples then underwent a charring cycle to simulate the carbonization of human bodies. The charring cycle exposed the bone and tissue samples to very high temperatures in an electric oven at 800°C for 12 hours; this was followed by a 12-hour cooling cycle.¹ As expected, every sample exhibited the morphological features of calcined bone with complete loss of soft tissues. The oven used was an electric industrial

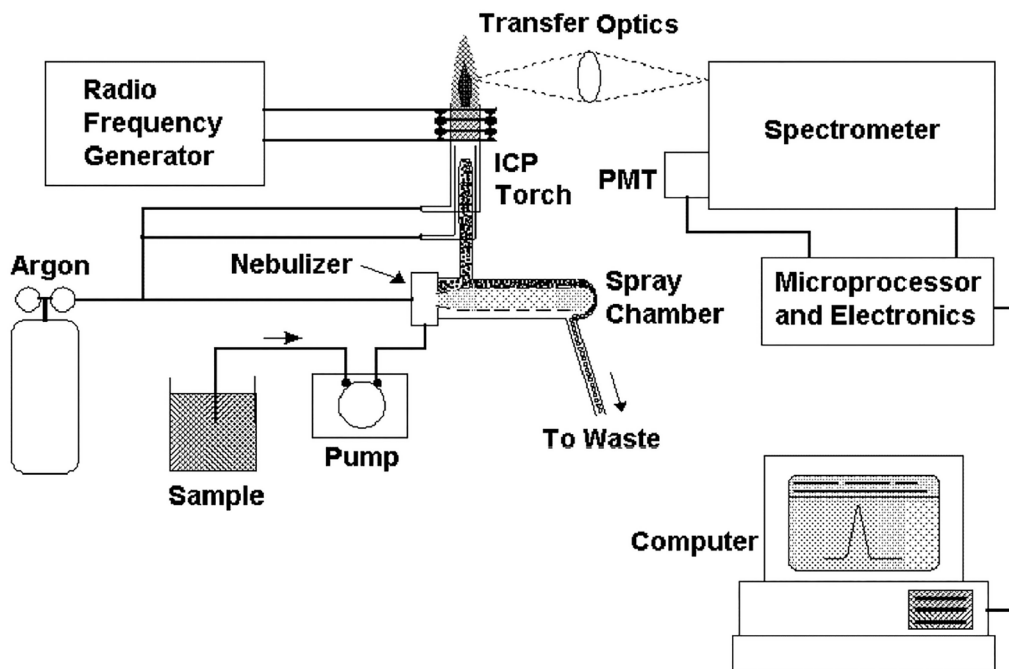


Figure 3: ICP-OES schematic and flowchart.⁶

machine (model Vega 150) with a capacity of 150 L (max temp. of 1280°C, with a rating of 10.0 kW and a tension of 230–400 V).¹ The heating cycle was performed on eight of the sixteen ribs.

After the charring cycle, the samples were taken out for sampling with a cotton swab soaked in 2mL of 30% nitric acid, sonicated in an ultrasonic bath for 10 minutes, and left at rest overnight. The sample was diluted in 5mL of DI water in which the cotton swab was rinsed to recover any leftover residues of the sample.¹ The ICP analysis was then performed using three calibration standards of 0.5 ppm, 2 ppm, and 5 ppm. The analysis was repeated three times for each sample. Overall, for each category composed of four ribs, two ribs were analyzed before and two ribs after the carbonization cycle. Two gunshot ribs were used as control samples, and all ribs underwent quantitative analysis via ICP-OES before and after the charring cycle. The metals analyzed under ICP-OES instrumental analysis that are under investigation for gunshot residue in charred bone and tissue samples are lead (Pb), antimony (Sb), barium (Ba), zinc (Zn), and copper (Cu).

The flowchart for the ICP-OES instrument in Figure 3 illustrates how the instrument analyzes inorganic atoms via spectroscopic analysis. In inductively coupled plasma-optical emission spectroscopy (ICP-OES), the sample is usually transported into the instrument as a stream of liquid sample. Inside the instrument, the liquid is converted into an aerosol through a process known as nebulization, with argon (Ar) gas as the carrier gas.

Pneumatic nebulizers make use of high-speed gas flows to create an aerosol.⁶ The sample aerosol is then transported to the plasma where it is ionized, atomized, and excited by the plasma.⁶ The plasma's maximum temperature is 10 000 Kelvin, which can excite atoms to higher electronic states and yield a very sensitive process. The excited atoms and ions emit their characteristic wavelengths, which are collected by a spectrometer device that sorts the emitted radiation into their individual spectra. A spectrum is an electromagnetic spectrum that is characteristic of individual atoms and elements based on their electronic configurations. A spectrum is, in effect, the fingerprint of the atom or element. The radiation is detected and turned into electronic signals that are converted into concentration information in computer software.⁶

All samples, both fresh and charred, resulted in significant concentrations of metallic residues compared with the control samples. The results of this GSR study indicated that the charring process did not significantly alter the values reported.¹ The ICP-OES analysis, via swabbing the wound site in the bullet entrance site, confirms that metallic residues from gunshot wounds can survive at very high temperatures (~800°C). The amount of surviving GSR after the carbonization process is always smaller, but in sufficient trace quantities to be detected with ICP-OES analysis.¹ Moreover, the study confirmed lead (Pb) as the main constituent of GSR, with significantly greater concentrations in samples shot with theunjacketed bullets. In the latter samples, a larger amount of residues was globally detected in both fresh and charred samples, probably due to the absence of the jacket, which allows the residues to spread and stick to the bone samples.

Table 4 lists the analyte concentrations for five elements/metals identified in GSR from both the full metal jacketed bullet projectile and theunjacketed lead bullet projectile. In Table 4 the results from the two types of ribs and two types of projectiles are organized into groups, each composed of four samples: from number 1 to 4, naked ribs and full metal-jacketed bullet (NF); from number 5 to 8, naked ribs and lead unjacketed bullet (NL); from number 9 to 12, dressed ribs and full metal-jacketed bullet (DF); and from number 13 to 16, dressed ribs and lead unjacketed bullet (DL).¹ The data in Table 4 display a decrease in metal concentration in charred bone samples compared to fresh tissue samples. Possible explanations for this may be the heat-induced volatilization in the electric oven to simulate the charring process that would occur in a real life situation, such as if a murderer sets the evidence on fire for the purpose of getting rid of it.

Table 5 provides a list of inorganic compounds that may contribute to GSR analysis via ICP-OES or ICP-MS. The compounds listed in Table 5 can be targeted to detect trace amounts of inorganic compounds that are characteristic of the bullet makeup. There are a large variety of mixtures for primers and casings. However, similar elements detected in previous studies using ICP-MS and ICP-OES comprise virtually all GSR components found in manufactured bullets and their fragments.

The results of this GSR analysis on cremated and burnt bone samples were quantified and shown to be very sensitive, as well as a reliable means of detection in burnt samples and degraded material. This instrumental method is optimum for analysis and detection of GSR trace metals on cremated bone and charred bone fragments. All samples, both fresh and charred, resulted in significant concentrations of metallic

Table 4: Concentration of absorbed metals from GSR on tissue samples for fresh and charred samples.¹

Samples	Metals (Concentrations in $\mu\text{g}/\text{cm}^2$)					
	Pb	Sb	Ba	Zn	Cu	
NF	Fresh					
	1	50	1.5	4.5	3.5	4.0
	2	36.3	0.8	8.6	2.2	2.2
	Mean	43.25	1.15	6.55	2.85	3.1
	Charred					
	3	3.28	0.145	1.78	2.31	2.41
NL	4	7.7	0.4	3.8	2.2	2.2
	Mean	5.49	0.273	2.79	2.255	2.305
	Fresh					
	5	1565	211	4.3	2.0	2.1
	6	1602	230	5.8	1.2	1.5
	Mean	1583.5	220.5	10.1	1.6	1.75
DF	Charred					
	7	540	12	11	1.5	0.9
	8	798	36	4.7	1.1	0.7
	Mean	669	24	7.85	1.3	0.8
	Fresh					
	9	19.5	3.5	25.0	3.0	4.5
DL	10	25.2	2.3	9.1	5.5	4.7
	Mean	22.35	2.9	17.05	4.25	4.6
	Charred					
	11	2.3	0.2	1.3	3.5	0.35
	12	4.8	0.7	2.9	3.9	2.1
	Mean	3.55	0.45	2.1	3.7	1.225
DL	Fresh					
	13	780	102	52.5	1.8	1.5
	14	478	225	4.2	2	0.1
	Mean	629	163.5	28.35	1.9	0.8
	Charred					
	15	995	12.3	34.2	0.8	0.8
DL	16	442	76	7.1	0.9	0.5
	Mean	718.5	44.15	20.65	0.85	0.65
	Negative control*	0.1	0.45	0.5	0.45	0.05
	Negative control†	0.2	0.11	0.23	0.97	0.02

NF, “naked” ribs, full metal-jacketed bullet.

*Before the charring cycle.

†After the charring cycle.

residues compared to the control samples. The results indicate that the charring process did not yield significant alterations in the values reported.¹ This study provides strong supporting evidence that the ICP-OES method of analysis, via swabbing the wound site or the bullet entrance, confirms that metallic residues from gunshot wounds can survive

Table 5: Inorganic compounds encountered in GSR. ²

Compound	Source of Compound
Aluminum	Primer/case
Aluminum sulfide	Primer mix
Antimony	Case/bullet
Antimony sulfide	Primer mix
Antimony sulfite	Primer mix
Antimony trisulfide	Primer mix
Arsenic	Case
Barium nitrate	Primer mix/propellant powder
Barium peroxide	Primer mix
Bismuth	Case
Boron	Primer mix
Brass	Case
Bronze	Bullet
Calcium carbonate	Propellant powder
Calcium silicide	Primer mix
Chromium	Bullet
Copper	Bullet jacket/primer cup/case
Copper thiocyanate	Primer mix
Cupro-nickel	Bullet jacket
Gold	Primer mix
Ground glass	Primer mix
Iron	Rust inside barrel, bullet
Lead	Bullet
Lead azide	Primer mix
Lead dioxide	Primer mix
Lead nitrate	Primer mix
Lead peroxide	Primer mix
Lead stannate (stypnate)	Primer mix
Lead thiocyanate	Primer mix
Magnesium	Primer mix
Mercury	Primer mix
Mercury fulminate	Primer mix
Nickel	Case
Nitrate	Black powder
Phosphorus	Case
Potassium chlorate	Primer mix
Potassium nitrate	Propellant powder/primer mix
Prussian blue	Primer mix
Red brass	Bullet jacket
Silicon	Primer mix
Sodium nitrate	Primer mix
Sodium sulphate	Propellant powder
Steel	Bullet core/case
Strontium nitrate	Primer mix
Sulphur	Primer mix/black powder
Tin	Primer mix
Titanium	Primer mix/Lead free primer mix
Tungsten	Bullet
Yellow brass	Bullet jacket/case
Zinc	Primer cup
Zinc peroxide	Primer mix
Zirconium	Primer mix

at very high temperatures (~800°C).¹ The study yielded trace amounts of GSR metal particulates which can be detected if the perpetrator attempts to burn away the body or the evidence.

Conclusions

The detection and quantification of traces of metals encountered in GSR are superior to qualitative examination under certain conditions. Qualitative difficulties resulting from fire or accelerant sources, and decomposition reactions arising from the environment can be addressed by conducting a quantitative analysis. The incorporation of an instrumental analysis is important for quantifying and identifying GSR in conditions unsuitable for qualitative analysis. As shown by the data in Tables 1-4 and Figure 2, GSR components can persist at very high temperatures and throughout decomposition processes. All data were generated via analytical procedures to calculate concentration values linear with respect to their calibrations. Calibration curves were not included in this research, and a few statistical problems exist in both studies involving ICP-MS and ICP-OES. The statistical problems are somewhat minor and can be corrected in future research. Future research goals in these studies include method validation of both analytical instrumentations in this research, as well as spectrophotometric and IR spectroscopic analysis on organic GSR before and after burning and/or decomposition. Lastly, future research goals in identifying GSR components include linking to an exact bullet make and manufacturer in order to help law enforcement agencies determine possible suspects based on gun owner registry. These studies provide evidence for the detection of GSR in severe conditions such as in burnt bone and tissue, and decomposed tissue samples. The detection and quantification of trace metals in GSR require specific conditions. Nonetheless, the results of these studies can help law enforcement agencies determine cause of death.

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