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Application of X-Ray Fluorescence (XRF) as a Sustainable, Non-Destructive Analytical Tool for Elemental Profiling in Biological Tissues: Insights from a Forensic Electrocution Case Study

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Abstract: ***Elemental analysis of biological tissues affected by electrical burns is essential for accurate forensic investigation of electrocution fatalities. In this study, a solvent-free and non-destructive approach based on portable X-ray fluorescence (XRF) spectroscopy was applied for elemental profiling of skin tissues. XRF enabled direct, in situ identification and quantification of elemental residues without chemical digestion, solvent use, or sample destruction, thereby ensuring both environmental sustainability and preservation of evidence. The case examined involved the electrocution of a female victim who reportedly received a fatal electric shock while handling a metallic desert cooler. Detailed XRF analysis of the lesion tissues revealed significant aluminium deposition at the suspected burn sites, which was absent in control tissues and inconsistent with the composition of the cooler and bucket recovered at the scene. These findings provided direct evidence of current conduction through aluminium contact, effectively challenging the preliminary investigative theory. The results highlight the forensic value of XRF as a rapid, sustainable, and reliable analytical tool, capable of supporting legal investigations while adhering to the principles of green analytical chemistry.***

Keywords**: Electrocution, X-ray fluorescence (XRF) spectroscopy, Solvent free analysis, green analytical chemistry, Forensic investigation, Elemental analysis, Tissue metallization.***

I. INTRODUCTION

Electric injury has become a frequent occurrence in forensic investigations, particularly in cases of fatal accidents. Due to the extensive integration of electricity into modern life, fatal electrocution incidents can occur across diverse settings and involve individuals from various socioeconomic backgrounds [1] . According to Eddleston et al., fatal electrocution is a common cause of unnatural death in several developing countries, especially in occupational or domestic cases due to the use of low-quality, informal electrical installations [2], [3]. The investigation in this manner is largely based on findings at crime scene. Different type of evidences were reported in this type of cases like wire, metallic piece, conductor (source of electrocution) injury of skin on victim [4], [5].

Electrocution deaths are perplexing in nature. A detailed post-mortem examination of an electrocution victim, however, should seek to establish the cause of death and circumstances surrounding the fatal accident [6], [7]. Electric injuries have been documented in 80% of fatalities resulting from electrocution [4], [8], [9]. Electrical burns are unique in that the wound at the skin surface may appear insignificant, while underlying damage can be extensive [10]. In order to evaluate this, a comprehensive approach involving various diagnostic methods is necessary. These methods include autopsy and vitroscopy, histopathological examination, biochemical changes within organs, and metallized skin analysis [5], [11]. Baumister and colleagues presented findings from radiological examinations using various methods, including whole-body postmortem CT (PMCT), PMCT-angiography (PMCTA), postmortem magnetic resonance tomography (PMMR), and PMMR-angiography (PMMRA). These imaging modalities revealed significant results, such as abnormalities in the intestinal mucosa and notable rhabdomyolysis in the extremities. [12]. Using routine macroscopic, scanning electron, and transmission electron microscopy, several authors have reported significant necrosis of the constriction band, lysis of myocardial cells, loss of striations, nuclear disappearance, and minimal vacuolization between the endothelium and the elastic internal membrane in coronary arteries [9], [13].

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Metallization, achieved through electrolysis, involves depositing metallic ions into the skin and subcutaneous tissues, creating a lasting indicator of electrocution observable even after extended periods. This phenomenon underpins forensic examinations of electrocution cases, with both traditional imaging and advanced analytical methods such as X-ray fluorescence (XRF) and scanning electron microscopy combined with energy-dispersive X-ray spectroscopy (SEM–EDS) being employed for their high sensitivity and specificity [7], [14], [15]. Early approaches relied on histochemical techniques like Perls' stain and Timm's method for detecting metals, [16], [17]. but there is a notable shift toward non-destructive methods enabled by EDS, which offer detailed elemental analysis and improved accuracy [13, 18]. Boracchi et al. observed limitations in conventional staining methods for copper and iron, contrasting with the heightened sensitivity of EDX, reinforcing its critical role in forensic investigations. [16]. XRF, as a widely utilized non-destructive technique, allows for rapid multi-element detection in skin tissues with minimal sample preparation, emphasizing its diagnostic purpose in electrocution cases. [19]. Recent findings demonstrate that XRF can successfully detect metallic microtraces even in the absence of visible charring and can apply non-invasive sampling methods like adhesive tapes for both postmortem analyses and living victims [20]. Further studies using microbeam X-ray fluorescence (m-XRF) have validated localized iron deposition from metallic wire contact, showcasing its diagnostic relevance [21]. Moreover, EDX is recognized for its rapid multi-element analysis capability, adept in identifying elements like Al, Sn, and Zn in tissues where traditional histological methods falter [22]. Collectively, these advancements illustrate the crucial integration of XRF and EDX methods in forensic anatomy, enhancing the accuracy of electrocution diagnosis, particularly in unusual or high-voltage scenarios [23]. The adoption of these cutting-edge techniques, alongside a thorough histopathological evaluation, significantly bolsters forensic evidence and understanding of metallization phenomena. The comparative analysis presented in Table I outlines the functionalities, benefits, and drawbacks of various elemental analysis methods, highlighting EDX's superiority in this context.

The paper advocates for a shift to analytical instruments for hypothesis validation and offers both quantitative and qualitative elemental analysis of human tissue. This analysis utilizes the EDX 7000 XRF spectrometer alongside stereomicroscopy to investigate the deposition of charred material and reveal the fundamental truths of the situation.

TABLE I

Comparative Overview of Methods, Operations, Advantages, and Disadvantages in Forensic Examination of Electrocution Cases

S.No.	Method	Operation	Advantage	Disadvantage
1	Autopsy	Autopsy and virtual autopsy	Provides complete internal and external examination; virtual autopsy is non-invasive	Conventional autopsy is invasive; virtual autopsy is expensive and requires advanced imaging
2	Histopathology	Conventional method	Detailed tissue analysis; gold standard for disease or trauma detection	Time-consuming; subjective interpretation
		SEM/TEM/Confocal microscopy	High-resolution imaging; 3D visualization of tissue architecture	Requires expensive instruments; technical expertise required
3	Biochemistry	IHC (Immunohistochemistry) / WB (Western Blot) / PCR	Sensitive and specific; allows detection of protein/DNA/RNA markers	Risk of contamination (PCR); requires specialized reagents and training
		FTIR-MSP (Fourier-transform infrared microspectroscopy)	Non-destructive molecular fingerprinting; good for trace analysis	Limited to certain compounds; overlapping peaks can cause interpretation issues
4	Metallization	Dry ash method and special staining method	Concentrates metallic content; useful for elemental detection in tissues	Destructive; possible loss of volatile elements
		AAS / SEM / ICP-MS / XRF	Highly sensitive and quantitative multi-elemental analysis	High cost; requires skilled operators and calibration

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II. CASE STUDY

The present investigation focuses on a case brought to the attention of FSL Rohini concerning the detection of metal in skin tissue, involving the reported death of a woman due to electrocution. According to the information provided in the forwarding letter by the police, a 32-year-old female was found died with a suspected history of electric shock. Upon arriving at the scene, law enforcement found the body on a cot, covered with a cloth. Further inquiries revealed that the victim had apparently suffered electric shock while pouring water into an electric metallic cooler (Fig.3), utilizing a metallic bucket (Fig.4).

A. Autopsy Findings

External Examination: The postmortem report submitted by the investigating officer in charge of the case stated that the dead had an average build, open eyelids, and seemed to be well-fed. Upon examination, congestion in conjunctivae, hypostasis on the back and limbs except the pressure area were observed and the rigour mortis had passed away. Furthermore, the abdomen displayed a greenish tint.

Additionally, the hands exhibited several external injuries characteristic of electrical contact. The palm of the left hand showed multiple contact wounds with underlying reddened subcutaneous tissues. A brownish electrical contact lesion was also present on the inner aspect of the left wrist at its dorsal surface, accompanied by similar tissue reddening. The right hand demonstrated multiple electrical contact wounds on the palmar surface, associated with tearing of adjacent tissues and subcutaneous congestion. A brownish contact wound was noted over the middle third of the dorsal left forearm, while another similar lesion was present on the lower third of the dorsal right forearm, both displaying reddened subcutaneous tissues. An abrasion with associated contusion was also observed on the left leg.

B. Histopathological Findings

As per histopathological report provided by investigation officer of concerned case the skin exhibited a stratified squamous epithelium with separation at the dermo-epidermal junction, along with blister formation within the epidermis. Additionally, there were signs of collagen degeneration in the dermal layer.

III.MATERIAL & METHOD

A. Sample Collection and Description

Four samples were submitted to the Forensic Science Laboratory (FSL), Rohini, Delhi, for comparative analysis of metallic components. The details of the samples are as follows:

1) Sample 1 (S1) (Fig. 1): Control skin tissue, preserved in 10% Formalin.
2) Sample 2 (S2) (Fig. 2): Suspected skin tissue, preserved in 10% Formalin.
3) Sample 3 (S3) (Fig. 3): Metallic cooler.
4) Sample 4 (S4) (Fig. 4): Metallic bucket.

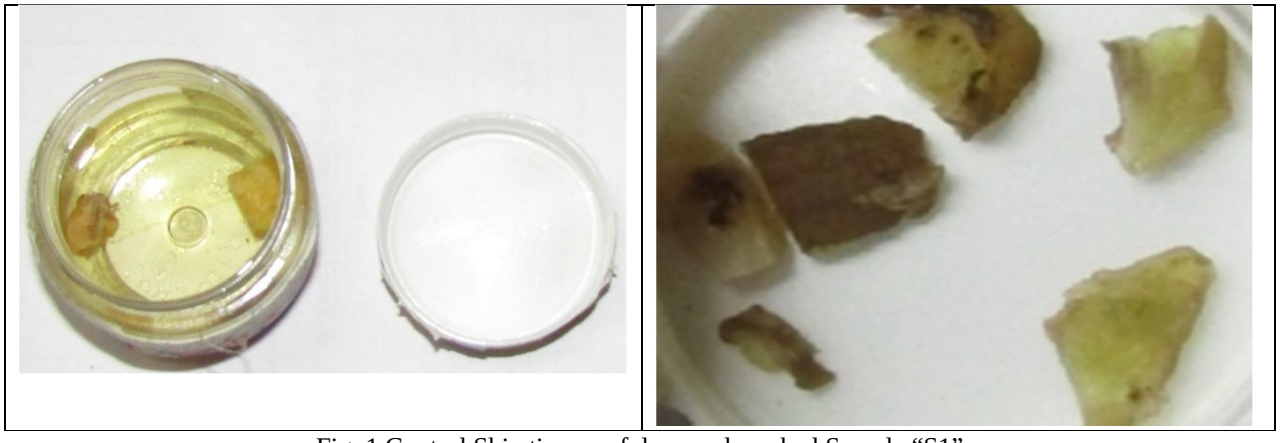


Fig. 1 Control Skin tissues of deceased marked Sample “S1”

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Fig. 2 Suspected skin tissue of deceased Sample “S2”

Samples from the metallic cooler (S3) and the metallic bucket (S4) were systematically collected from the suspected location using a sterile metal scraper to ensure contamination-free collection for subsequent forensic analysis.



Fig. 3 Metallic cooler Sample “S3”



Fig. 4 Metallic bucket Sample “S4”

B. Stereomicroscopic Examination

The skin tissue samples (S1 and S2) were examined for gross morphological changes. Subsequently, the tissues, previously preserved in preservatives, were carefully dried at ambient room temperature in sterile Petri dishes. The samples were subjected to a detailed physical examination using a stereo-microscope (Olympus Optical Co. Ltd., Model SZX-ILLD200). Observations were carried out at varying magnifications to assess surface morphology, texture, and physical alterations. The stereo-microscope was operated under reflected light mode, which enabled a three-dimensional visualization of the sample surfaces. Multiple magnifications were employed sequentially, beginning with low-power scans to identify overall features and progressing to higher magnifications for closer inspection of fine structural details. Digital images were documented for comparative purposes.

C. Elemental Analysis by X-Ray Fluorescence (XRF) Spectroscopy

Elemental analysis for metal detection in all samples (S1–S4) was performed using a Shimadzu 7000EDX X-ray Fluorescence (XRF) spectrometer. The instrument was operated under the following parameters:

1) Target: Rhodium anode
2) Operating voltage: 40 keV
3) Operating current: 15 keV
4) X-ray path: 3 mm collimator, vacuum
5) Detector: Silicon (lithium)
6) Measurement time: 300 seconds per sample

Dried skin tissue samples were placed directly onto the sample stage of the XRF spectrometer without additional preparation. Spectra were recorded for each sample, and the presence of metallic elements was documented for comparative analysis. The elemental composition of all four samples, S1(Control area of lesion skin change), S2(suspected black area of lesion skin), S3(Metallic cooler) and S4(Metallic bucket) were recorded.

IV. RESULTS

Naturally, human body consist of number of elements and each element has its own significance inside the body to maintain its functionality [24], [25]. So, in this condition it is great dilemma to find out the extraneous metallic composition in body tissues due to electrocution. In this study we represent the results of analytical techniques to ascertain the above issue. Microscopic examination of the suspected skin tissue samples (S2) demonstrated distinct morphological alterations, as illustrated in Fig.5. Under high magnification, blackish deposits on the tissue surface was observed with deep indentations filled with similar black material suggesting potential sites of thermal injury associated with electrical burns. Comparable electrical injuries with deep tissue involvement have been reported previously [26]. In contrast, control skin tissue exhibited a uniform yellowish appearance without black indentations, as illustrated in Fig. 6.

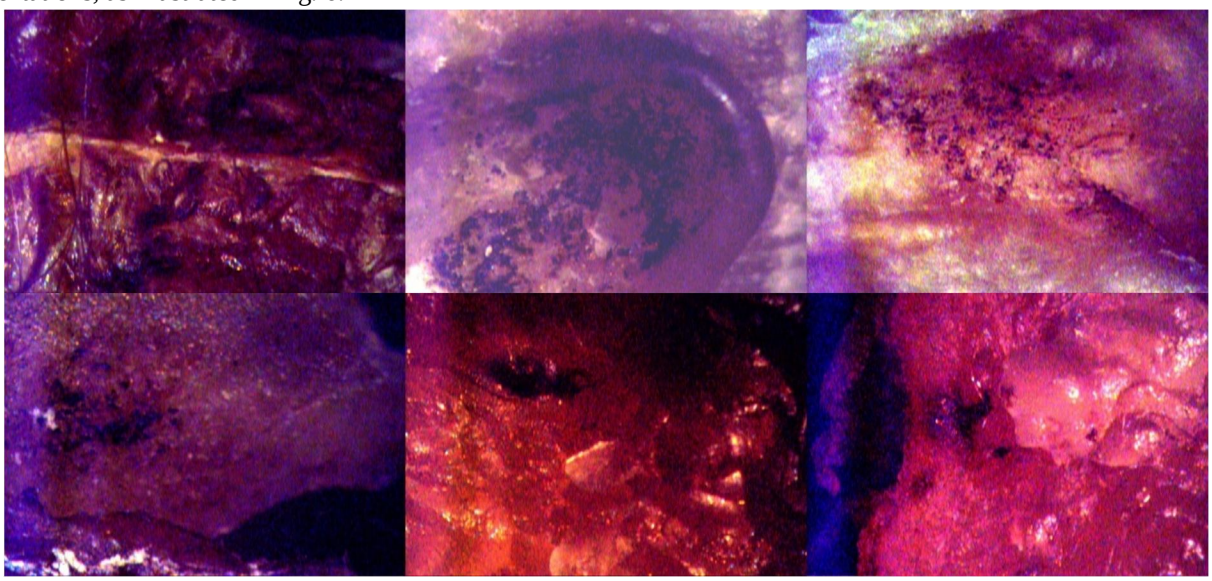


Fig. 5: Microscopic analysis of suspected skin tissue samples (S2)

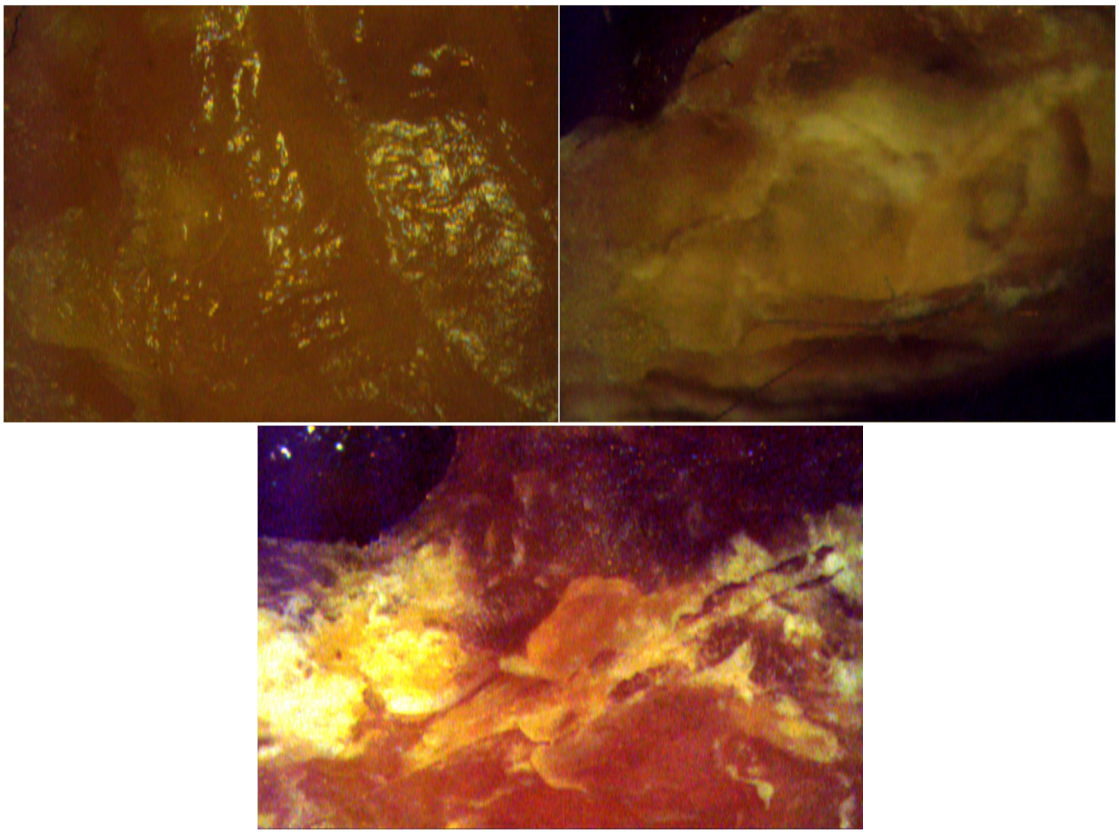
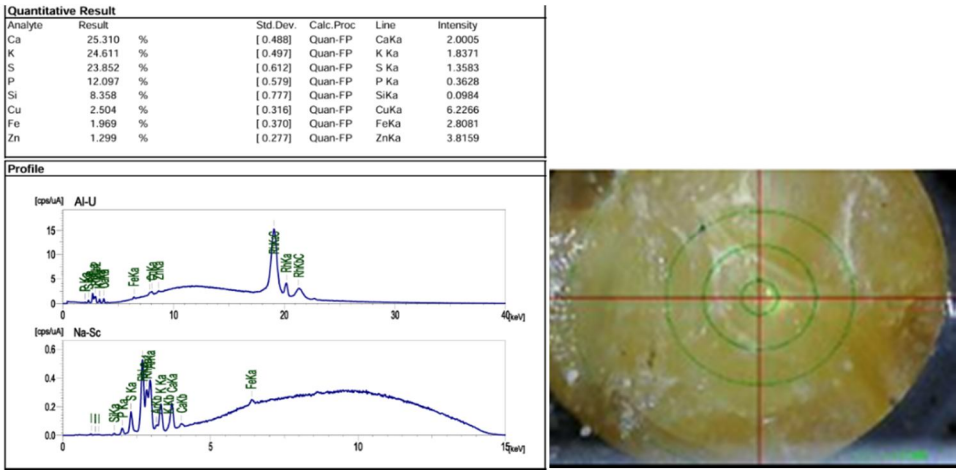


Fig. 6: Microscopic analysis of control skin tissue samples (S1)

Elemental analysis conducted through XRF spectroscopy yielded additional insights, with the results presented in Figs. 7–10. Control skin tissue (S1), free from any black deposition, exhibited characteristic peaks for biologically relevant elements such as calcium (Ca), potassium (K), sulfur (S), and phosphorus (P). In contrast, XRF spectra recorded from regions containing black deposits demonstrated an additional prominent peak corresponding to aluminum (Al Kα ~1.49 keV). Moreover, minor peaks for iron (Fe) and trace amounts of zinc (Zn) were also detected in the suspected lesions (S2). These peaks were absent in control tissues, indicating the introduction of extraneous metallic elements into the skin, potentially due to external contact or contamination.

Analysis of the metallic items submitted for investigation revealed further contextual information. The bucket surface exhibited prominent peaks for iron (Fe), chromium (Cr), and manganese (Mn), consistent with galvanized steel composition, whereas the cooler surface showed major peaks for iron (Fe), zinc (Zn), and copper (Cu), with no detectable aluminum (Al) on any of the metallic surfaces



Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
Ca	25.310	%	[0.488]	Quan-FP	CaKa	2.0005
K	24.611	%	[0.497]	Quan-FP	K Ka	1.8371
S	23.852	%	[0.612]	Quan-FP	S Ka	1.3583
P	12.097	%	[0.579]	Quan-FP	P Ka	0.3628
Si	8.358	%	[0.777]	Quan-FP	SiKa	0.0984
Cu	2.504	%	[0.316]	Quan-FP	CuKa	6.2266
Fe	1.969	%	[0.370]	Quan-FP	FeKa	2.8081
Zn	1.299	%	[0.277]	Quan-FP	ZnKa	3.8159

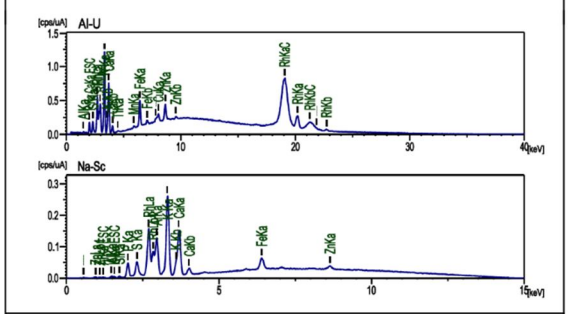
Fig.7 XRF graph of S1 (Control area of lesion skin)

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Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	31.581	%	[0.321]	Quan-FP	K Ka	2.6376
Ca	19.477	%	[0.239]	Quan-FP	CaKa	1.5482
Al	18.106	%	[1.158]	Quan-FP	AlKa	0.0468
P	14.678	%	[0.375]	Quan-FP	P Ka	0.4005
S	8.800	%	[0.193]	Quan-FP	S Ka	0.4707
Si	3.873	%	[0.456]	Quan-FP	SiKa	0.0358
Fe	1.897	%	[0.029]	Quan-FP	FeKa	2.8632
Zn	0.677	%	[0.027]	Quan-FP	ZnKa	2.1541
Cu	0.409	%	[0.029]	Quan-FP	CuKa	1.1003
Tl	0.312	%	[0.075]	Quan-FP	TiKa	0.1267
Mn	0.190	%	[0.044]	Quan-FP	MnKa	0.2110

Profile



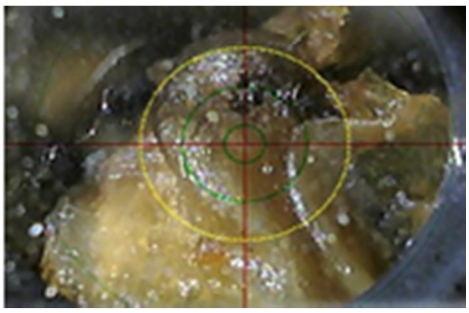
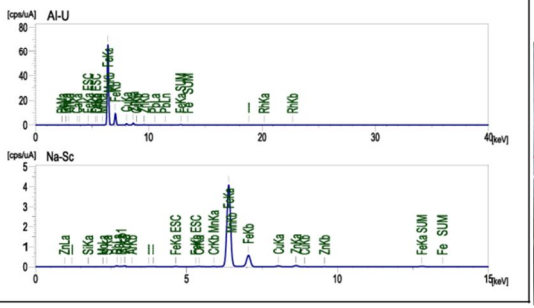


Fig.8 XRF graph of S2 (suspected black area of lesion skin)

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
Fe	90.966	%	[0.222]	Quan-FP	FeKa	482.3895
Zn	4.319	%	[0.063]	Quan-FP	ZnKa	13.0928
Cu	3.262	%	[0.058]	Quan-FP	CuKa	8.4138
Si	0.704	%	[0.159]	Quan-FP	SiKa	0.0101
S	0.411	%	[0.051]	Quan-FP	S Ka	0.0516
Mn	0.186	%	[0.014]	Quan-FP	MnKa	0.9606
Cr	0.086	%	[0.008]	Quan-FP	CrKa	0.6027
Ca	0.066	%	[0.022]	Quan-FP	CaKa	0.0982

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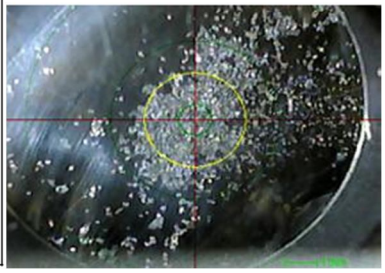
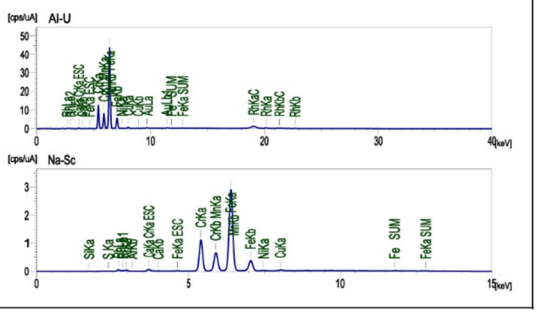


Fig.9 XRF graph of S3 (Metallic cooler)

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
Fe	69.708	%	[0.233]	Quan-FP	FeKa	313.4960
Cr	14.533	%	[0.094]	Quan-FP	CrKa	86.5377
Mn	8.857	%	[0.068]	Quan-FP	MnKa	47.2099
Cu	1.672	%	[0.035]	Quan-FP	CuKa	5.2500
Si	1.627	%	[0.170]	Quan-FP	SiKa	0.0300
Au	1.532	%	[0.087]	Quan-FP	AuLa	2.8526
Ca	1.279	%	[0.028]	Quan-FP	CaKa	0.6863
Ni	0.480	%	[0.043]	Quan-FP	NiKa	1.2570
S	0.313	%	[0.050]	Quan-FP	S Ka	0.0489

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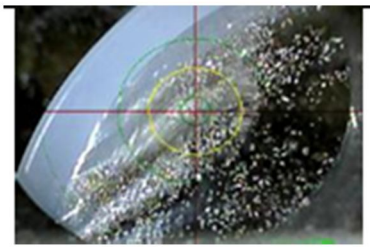


Fig.10 XRF graph of S4 (metallic bucket)

XRF spectroscopy results, summarized in Table II and Fig. 11, demonstrate aluminium deposition in the marked suspected skin sample, while control tissues and major metallic surfaces lacked such findings, thereby supporting the interpretation of metallization through direct contact with an aluminium surface. These findings underscore the forensic value of XRF in detecting elemental alterations in skin and reconstructing the source and sequence of electrical injuries. Similar cases of homicidal electrocution often disguised as accidents have been reported, including one involving a 46-year-old male with toe burns, head trauma, nail avulsion, and ligature marks, where histopathology confirmed electrocution. Such observations highlight the importance of correlating injury distribution, restraint or torture marks, and scene evidence to differentiate homicidal electrocution from accidental fatalities [27].

TABLE II

Comparative table of elemental composition in lesion skin (S1), control skin (S2), cooler sample (S3), and bucket sample (S4). "–" indicates no peak above background. The elements present in concentration more than 1% are constituted as major element and shown in comparative table

Element	Lesion skin (S1)	Control skin (S2)	Cooler sample	Bucket sample
Calcium	19.4	25.3	–	–
Phosphorus	14.6	12	–	–
potassium	31.5	24.6	–	–
Aluminum (Al)	+18.1	–	–	–
Iron (Fe)	+(baseline)1.8	+ (baseline) 1.9	+ 90.96%	+ 69.7
Zinc (Zn)	+(baseline)	+(baseline) 0.6	+ 4.3	–
Copper (Cu)	+(baseline)	+ (trace) 0.4	+(3.2)	+1.6
Chromium(Cr)	–	–	–	+14.5
Mangnese(Mn)	–	–	–	+8.8

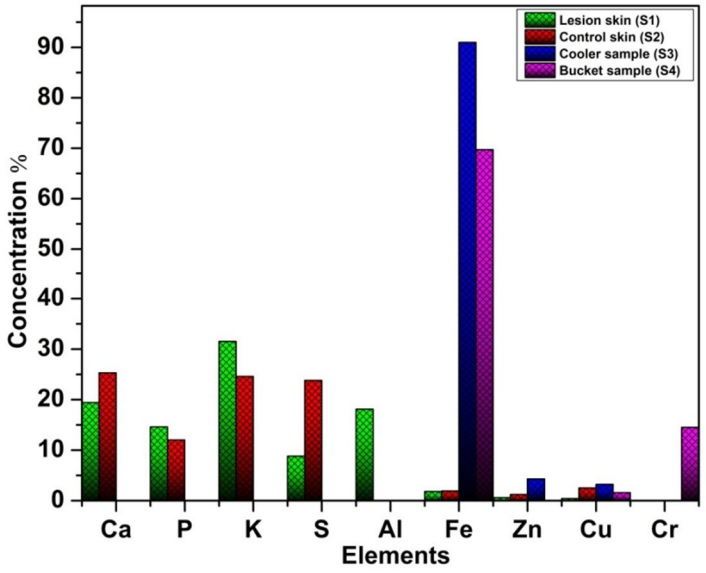


Fig 11 Bar graph representation of the data representing in table II illustrating variations in elemental concentrations across biological and metallic evidence.

V. DISCUSSION

This case demonstrates the utility of XRF in confirming electrocution. Aluminium was detected in lesion skin but not in control tissue or reference objects, supporting current flow through contact with an aluminium surface. Such metallization is a recognized hallmark of electrical injury and was not attributable to environmental contamination.

Prior studies support this interpretation. Wang et al. reported that microbeam XRF identified iron in a DC-shock current mark matching the scene wire [21]. Similarly, Tambuzzi et al. demonstrated that portable XRF can detect steel-derived elements (Fe, Mn, etc.) in contact marks from energized rails [20]. Our findings extend this approach by showing that XRF can be applied without complex sample preparation, enabling on-site scanning of tissue and inanimate evidence. Its rapidity and portability make it a practical adjunct to autopsy, serving as a non-destructive chemical "stain" for electrocution marks. Tambuzzi et al. further noted that even adhesive tape applied to lesions can capture metallic residues for XRF, opening possibilities for live victim evaluation [20]. In our practice, strict precautions were taken avoiding washing, contaminated glassware, or metallic contact to prevent false deposits, given the high sensitivity of XRF to trace metals.

There are, however, limitations. XRF only detects elements with atomic number >10, and aluminium (Z=13) lies near its lower sensitivity threshold. While our XRF unit readily detected it at high concentrations, quantification is challenging, especially on uneven skin surfaces. Tambuzzi et al. observed similar issues when attempting to quantify Fe in irregular samples [20]. For this reason, our analysis focused on presence/absence and relative intensities rather than absolute quantitation.

Differential diagnosis was also considered. Thermal, sharp, or blunt injuries can produce burn-like lesions but do not cause metal deposition. The coexistence of electrical burn histology with XRF-detected aluminium supports electrocution as the most plausible cause. As Meshram et al. emphasize, unusual burn patterns (e.g., atypical locations) should raise suspicion of foul play [20]. That caution applies here as well the presence of a cooler and bucket at the scene does not align with the metallization observed across multiple contact lesions in a restrained victim, findings more consistent with a non-accidental electrocution scenario.

Despite these limitations, this case highlights the potential of XRF in forensic investigation. Although not yet standardized in forensic protocols, XRF provides rapid, non-destructive elemental profiling with minimal preparation. With careful application and interpretation, it can deliver definitive support in electrocution cases, complementing histopathology and scene analysis

A. Environmental Implications

A notable advantage of the present approach is its alignment with green analytical chemistry. Conventional elemental analysis of burn tissue often involves acid digestion, atomic absorption spectroscopy, or ICP-based methods, which require toxic solvents, generate hazardous waste, and destroy valuable forensic evidence. In contrast, XRF avoided solvents and destructive sample preparation, reducing chemical waste and preserving the integrity of biological tissue. This solvent-free, low-waste method not only supports environmentally sustainable forensic practice but also ensures that evidentiary material remains intact for complementary analyses.

VI. CONCLUSION

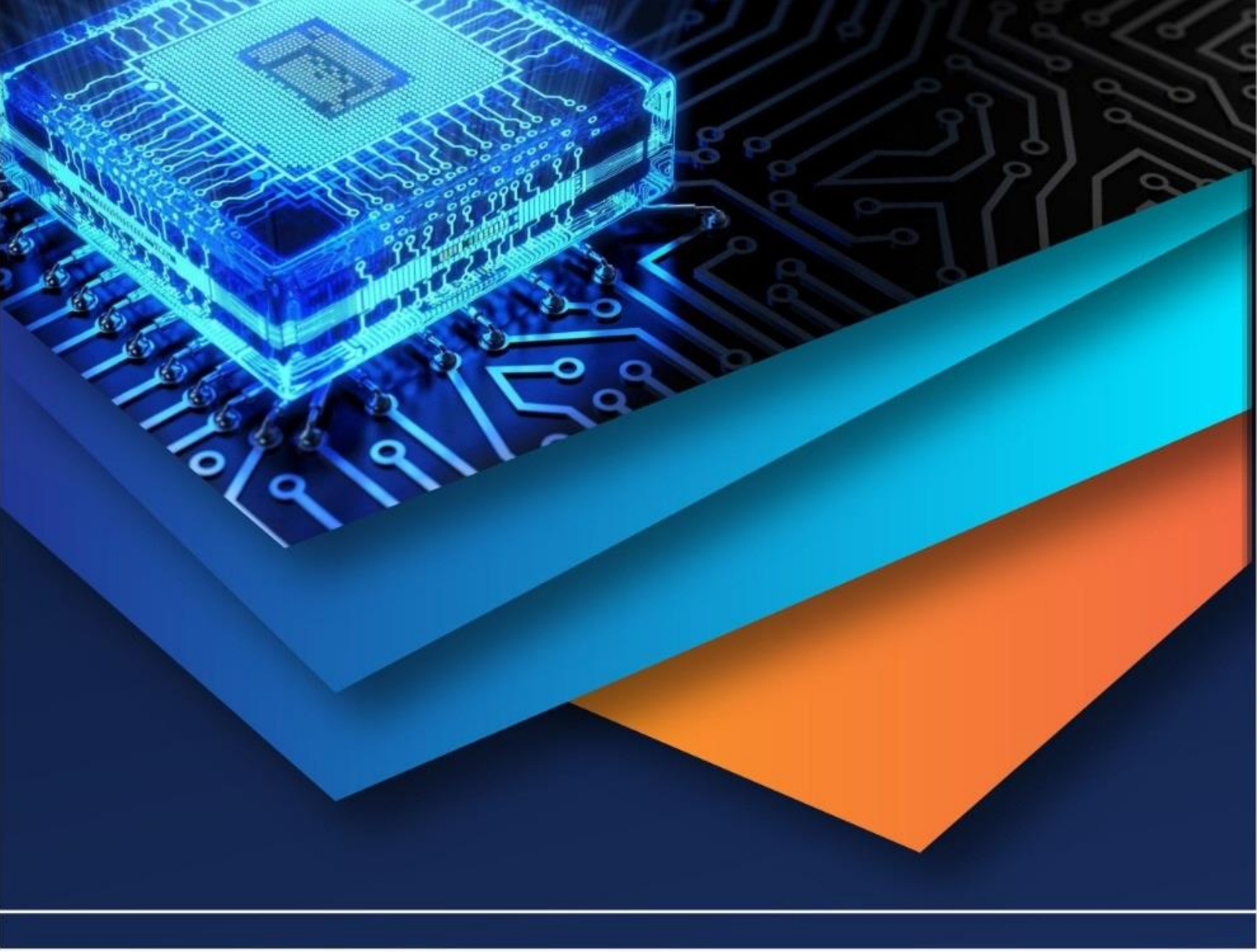
This study highlights the dual forensic and environmental advantages of applying X-ray fluorescence (XRF) in electrocution investigations. Unlike conventional elemental analysis techniques that rely on acid digestion, solvents, or destructive preparation, XRF is solvent-free, generates negligible chemical waste, and preserves biological evidence for complementary testing making it a green analytical alternative aligned with sustainable forensic science. The case findings demonstrated aluminium exclusively in lesion tissue and not in control samples, confirming metallization from direct contact with an aluminium-bearing surface. This evidentiary value is particularly significant given that traditional diagnostic techniques, such as histopathology, cannot reveal elemental composition. The high sensitivity of XRF, however, also underscores the importance of strict exhibit management to avoid contamination. Looking forward, integration with complementary methods such as scanning electron microscopy (SEM) can enhance morphological and compositional resolution, strengthening the ability to distinguish contact-induced metallization from electrical damage. The combined application of XRF and microscopy thus provides a powerful, environmentally responsible, and scientifically rigorous framework for reconstructing injury mechanisms and supporting medico-legal conclusions in cases of suspected electrocution.

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