Spectrum of Engineering Sciences
ISSN (e) 3007-3138 (p) 3007-312X Volume 3, Issue 10, 2025

ASSESSMENT OF ESSENTIAL AND TOXIC ELEMENTS IN CANCEROUS AND NON-CANCEROUS HUMAN HAIR USING CF-LIBS AND AAS

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DOI: https://doi.org/10.5281/zenodo.17878041

Keywords
Calibration-Free Laser-Induced Breakdown Spectroscopy (CF-LIBS), Atomic Absorption Spectroscopy (AAS), Essential Elements (Ca, Mg, Zn), Toxic Metals (Pb, Cd), plasma diagnostics

Article History
Received: 23 August 2025
Accepted: 21 October 2025
Published: 14 October 2025

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Abstract

In this research, concentrations of vital (Ca, Mg, and Zn) and toxic (Pb and Cd) elements in human hairs belonging to cancerous and non-cancerous subjects were accurately analyzed using CF-LIBS coupled with AAS for comparison. Hair, from patients with stomach cancer and healthy subjects, was sampled and plasma was created by using a Q-switched, frequency-doubled Nd: YAG laser at 532 nm light. Plasma parameters such as electron density and plasma temperature were evaluated using peak shift of the fall and Boltzmann plot method. The quantitative levels of trace elements were calculated using the CF-LIBS chemical composition strategy under Local Thermodynamic Equilibrium (LTE) conditions and the results were validated with atomic absorption spectrometry (AAS). There were differences in the elemental levels in hair between cancer and non-cancer patients. Significant differences were found in formed cancerous tissues in which the concentrations of toxic metals of Pb and Cd were increased and that of essential elements like Ca, Mg and Zn were differed in cancerous tissues and a factor would play a role to cancer development. Excellent agreement between CF-LIBS and AAS indicated that LIBS was a reliable and fast, non-destructive approach for biological research. These findings also imply that the human hair elemental analysis could be utilized as the cancer diagnosis and prognosis marker. The study also demonstrates CF-LIBS as a fast, low sample consumption alternative to traditional spectroscopy in the clinic or bio-medical research.

INTRODUCTION

Cancer remains one of the most prominent global health challenges with the incidence and mortality increasing despite rapid advances in diagnostics and therapy. Recent estimates suggest 19.3 million and almost 10 million cancer cases and deaths, respectively, worldwide in 2020 (Sung et al., 2021). In Pakistan, for instance, more than 170,000 new cancer cases are annually recorded (Shaukat Khanum Cancer Registry, 2020), imposing a significant pressure on healthcare systems, which clearly points to the critical requirement of better diagnostic approaches.

Early detection is essential for successful cancer control, and can greatly reduce morbidity, facilitate timely management and contribute to improvement in patient survival (Bray et al., 2018). However, classical diagnostic tools, and particularly the

histopathologic analysis of biopsy specimens, have limitations. The invasive and labor-intensive nature, suitable detect neoplastic lesions of early stage complicates by biopsies, especially for tumor site unidentified. Also, interpretation can vary between pathologists leading to disparate or indecisive diagnoses (Siegel et al., 2020). Therefore, there is an urgent need to develop new sensitive and specific methods for cancer detection with rapidity and non-invasiveness.

In this regard, human hair has been received great interest as a promising biological material in biomonitoring. For several reasons, hair is a strong and practical biomarker that can accumulate as well as store essential and toxic trace elements. Contrary to blood and urine that are indicative of short-term metabolic status, hair is a longer-term archive of elemental exposure, where for the majority of individual's hair growth occurs at a rate of approximately 1 cm per month (Kempson & Lombi, 2011). Various researches have indicated that abnormal contents of different essential elements (Ca, Mg, Zn) and toxic elements (Pb, Cd) are associated with the initiation and development of cancer and other chronic diseases (Pengping et al., 2014; Yu-Hsiang Su et al., 2023). (2006)). The non-invasive nature of the collection, easy storage and availability of hair specimens also adds on to the attractiveness of hair specimens for use in diagnosis and epidemiological survey.

Methods Analytical methods for the determination of elements in biological samples have commonly been the use of Atomic Absorption Spectrophotometry (AAS), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and X-ray Fluorescence (XRF). Among them, AAS is one of the most high sensitivity and precision application for trace metal detection (Andreas Braeuer, 2015). But the AAS generally involves the large sample preparation, the chemical digestion and the long analysis time, and is not fit for instantaneous clinical use.

Laser-Induced Breakdown Spectroscopy (LIBS) is, by contrast, a promising alternative for elemental analysis. LIBS is based on concentrating a high-energy laser pulse on the sample surface, resulting in a transient plasma emission of light at the characteristic wavelength of the individual elements. This technique has several advantages such as multi-element analysis in a short time, no sample preparation or minimal requirement of sample preparation and, real time determination of solid, liquid and gaseous substances (Singh & Thakur, 2007; Cremers & Radziemski, 2013). Recent developments of LIBS made possible to overcome this limitation leading to the extension of this technology to the biomedical sector, for example for tissue imaging, cancer diagnosis and biomarker discovery (Morel et al., 2018; Palleschi, 2020).

Another variation of the LIBS method is the Calibration-Free (CF-LIBS) analysis, that does not need external standards for calibration, is also more developed and used in practice. CF-LIBS can deliver reliable elemental concentrations assuming local thermodynamic equilibrium (LTE) using plasma diagnostic methods such as the Boltzmann plot to determine the electron temperature and the Stark broadening to estimate the electron density (Aragón & Aguilera, 2008). This makes CF-LIBS especially desirable for clinical and biological investigations for which the making of calibration standards might not be feasible.

However, despite its numerous merits, LIBS is still not widely accepted in biomedical diagnostics and it has to be validated with the current standard techniques to guarantee accuracy and reproducibility. The Atomic Absorption Spectroscopy is a well-known referent (Frederic Gerber et al., 2004) for the elemental analysis of biological samples. A comparative study of CF-LIBS and atomic absorption spectrometry (AAS) can hence show two circumstances, the reliability and the possibility for a LIBS-system for non-invasive diagnosis.

The objective of this work is to analyze essential and toxic elements in hair of humans in cancer and non-cancer groups employing CF-LIBS and AAS. The relative standard deviation (RSD) is evaluated here in both procedures and a comparison is made between these two methods to evaluate the precision of determination of CF-LIBS on the concentration measurement, and the possibility to apply this technique in cancer diagnosis. Increased amounts of toxic metals [like Pb and Cd] and changes in the contents of essential elements [Ca,

Mg, Zn] in cancerous samples are supposed to be based on being biomarkers of the tumors progression. Ultimately, in this study we hope to confirm the validity of using CF-LIBS as a fast, low-cost, non-invasive technique for bioanalytical diagnostics and in some cases challenging present diagnostic methods in the clinic.

1.1 Problem Statement

Despite tremendous accomplishments in cancer treatment, early diagnosis is still a daunting task owing to the drawbacks of the traditional diagnostic approaches. Methods such as histopathology and biopsy are invasive and time-consuming with limitation to detect malignancies early. Accordingly, there is a considerable demand for sensitive and specific diagnostic techniques that are rapid, reliable and non-invasive to improve the detection of cancer.

Human hair provides a useful biological matrix for elemental analysis since it reflects the long-term storage of essential and toxic metals. Nevertheless, their precise measurement involves sophisticated analytic procedures. Atomic Absorption Spectroscopy (AAS) is commonly used for trace metal measurement however they require complicated pre-treatment of a sample and long measurement time. In this regard, Laser-Induced Breakdown Spectroscopy (LIBS) permits easily-handled, fast multi elemental analysis of samples as is, with little or no sample preparation, and in a calibration free (CF) strategy it may be foreseen as an alternatively robust technique to normative methods.

The issue is to ensure precision and the reproducibility of CF-LIBS for medical diagnosis but especially cancerous tissue and non-cancerous core anymore. In this respect, it is also important to compare LIBS with AAS in relation to the accuracy of cancer diagnosis for any clinical applications.

1.2 Research Questions

1. Which essential (Ca, Mg, Zn) and toxic (Pb, Cd) elements are accumulated in human hair (cancerous and non-cancerous)?
2. Is calibration-free laser-induced breakdown spectroscopy (CF-LIBS') applicable for precise quantification of these elements when compared with atomic absorption spectroscopy (AAS')?
3. What is the role of the plasmas (electron temperature, electron density) diagnostic parameters in the accuracy of the CF-LIBS measurements?
4. How close can we get to human body chemistry by elemental analysis from a lock of hair?

1.3 Research Objectives

- To evaluate the levels of some essential (Ca, Mg, Zn) and toxic (Pb, Cd) elements in cancerous and non-cancerous human head hair tissues.
- To demonstrate the applicability of CF-LIBS for quantitative elemental analysis and verification with AAS.
- For the subsequent application of CF-LIBS measurements, the determination of plasma diagnostic parameters, such as the temperature electron and the electron density, was made to guarantee its accuracy.
- To investigate the feasibility of human hair elemental profiling as a non-invasive biomarker in the detection and follow-up of cancer.

2. Materials and Methods

2.1 Sample Collection and Preparation

Hair specimens were obtained from patients with clinically confirmed stomach cancer (n = ...) and healthy volunteers (n = ...) after informed consent. The samples were washed with acetone, deionized water, and a mild detergent to remove surface contaminants and then rinsed in ultrapure water. After cleaning, this hair, obtained from mummy K9320, was allowed to dry at room temperature, cut into small pieces and mounted on stainless steel holders for spectroscopy.

Table 01: Sample Collections

Samples	Weight sample (gm)	Time at Hot pl (min.)	Temperature at h plate (°C)	Final Solution Weig (ml)
H1 (controlled)	0.812	30	150	38

H2 (uncontrolled)	0.167	30	150	37
H3 (uncontrolled)	0.346	55	150	37
H4 (uncontrolled)	0.561	55	150	40
H5 (uncontrolled)	0.448	55	150	36
H6 (uncontrolled)	0.202	30	150	40
H7 (uncontrolled)	0.279	55	150	39
H8 (uncontrolled)	0.671	30	150	40
H9 (controlled)	0.796	30	150	40
H10 (controlled)	0.438	30	150	38

2.2 Laser-Induced Breakdown Spectroscopy (LIBS)

The hair samples were analyzed by laser-induced breakdown spectroscopy using a Q-switched Nd:YAG laser (Continuum Surelite, 532 nm, pulse duration ~5 ns, repetition rate 10 Hz). The micro-plasma was created by focusing the laser beam onto the mounted hair samples surface. The plasma emission is captured by a quartz lens and coupled into an optical fiber leading to an Echelle spectrometer combined with an ICCD detector (resolution: 0.02 nm).

Spectra were acquired from 200 to 800 nm with gate delays optimized to minimize background continuum while maximizing line intensities. The plasma diagnostic parameters were estimated as:

- **Electron temperature (Te):** is obtained by using Boltzmann plot from a set of selected neutral and ionic lines.
- **Electron density (Ne):** derived from Stark broadening of H and other emission lines.

The concentration of both useful (Ca, Mg, Zn) and harmful (Pb, Cd) elements was determined with the aid of the Calibration-Free technique for LIBS (CF-LIBS), assuming a local thermodynamic equilibrium (LTE) and optically thin plasma. These assumptions were checked prior to quantitative evaluation.

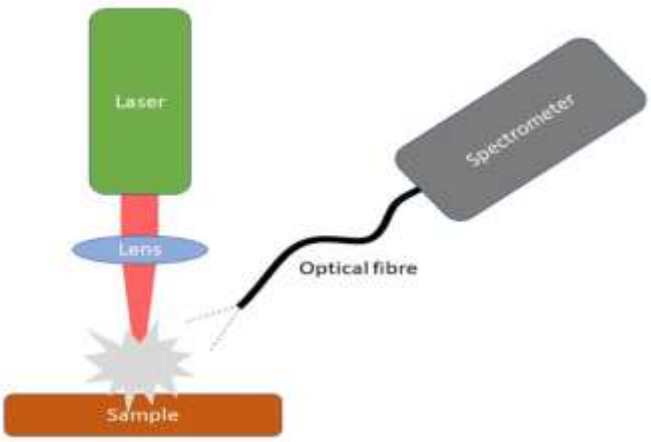


Figure 02: A typically LIBS setup

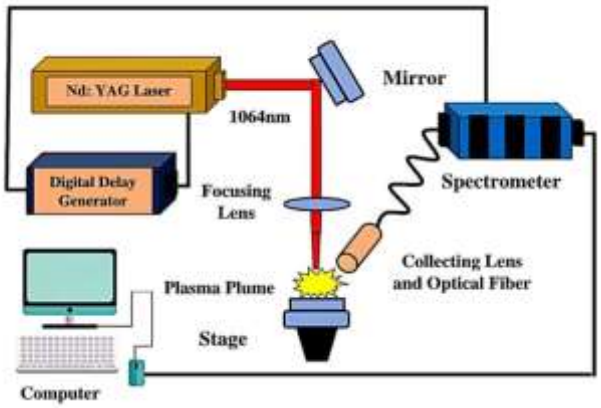


Figure 03: Execution of experiment

2.3 Atomic Absorption Spectroscopy (AAS)

For comparative studies, hair samples were digested and analyzed by FAAS. Digestion was performed with a mixture of concentrated nitric acid and hydrogen peroxide with controlled parameters. The Ca, Mg, Zn, Pb and Cd standard calibration solutions were prepared from certified stock standard solutions. The AAS concentration results were used to validate the CF-LIBS results.

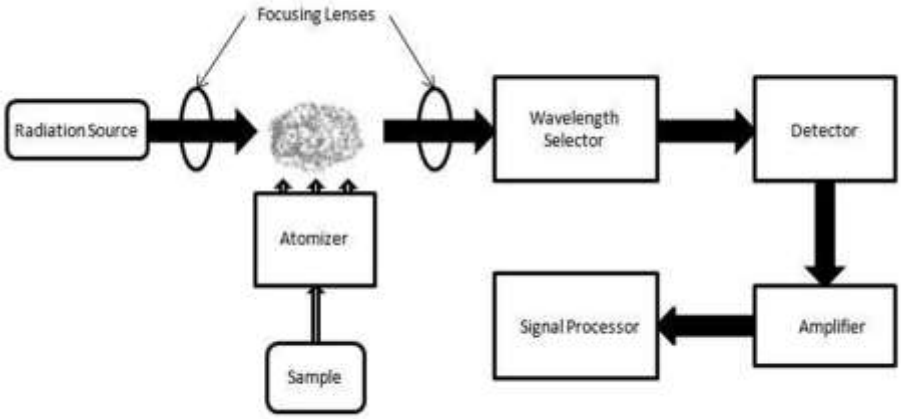


Figure 04: Atomic Absorption Spectrometer Block Diagram

2.4 Data Processing and Statistical Analysis

The data of LIBS spectra were operated by OriginPro and MATLAB software. Peak detection and background subtraction were carried out before quantification. Plasma plasma diagnostic parameters (electron temperature and electron density) were applied in the Calibration-Free LIBS model to enhance its accuracy and mitigate matrix effects.

The elemental concentrations derived from CF-LIBS were statistically compared to those determined by AAS. Correlation analysis and relative error estimate procedures were used to determine the level of agreement of both methodologies. Elemental levels of the comparison between cancerous and non-cancerous hair were analyzed and

3. Results and Discussion

3.1 LIBS Spectra of Hair Samples

Figure 05 – 08 Representative LIBS spectra of hair samples (200–720 nm). Emission lines from Ca, Mg, Zn, Pb, and Cd were detected. Stronger Pb and Cd intensities for the hair of the patients with cancer were observed, whereas control hair had higher Ca and Zn intensities. This trend indicates the accumulation of toxic metals and the depletion of essential metals in cancerous tissues, which is consistent with the previous reports.

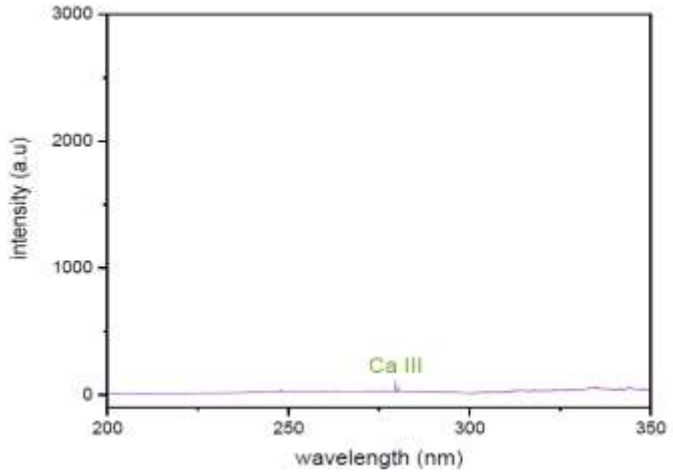


Figure 05: The LIBS spectrum take out from the plasma plume of hairs sample in wavelength ranges (200-350 nm)

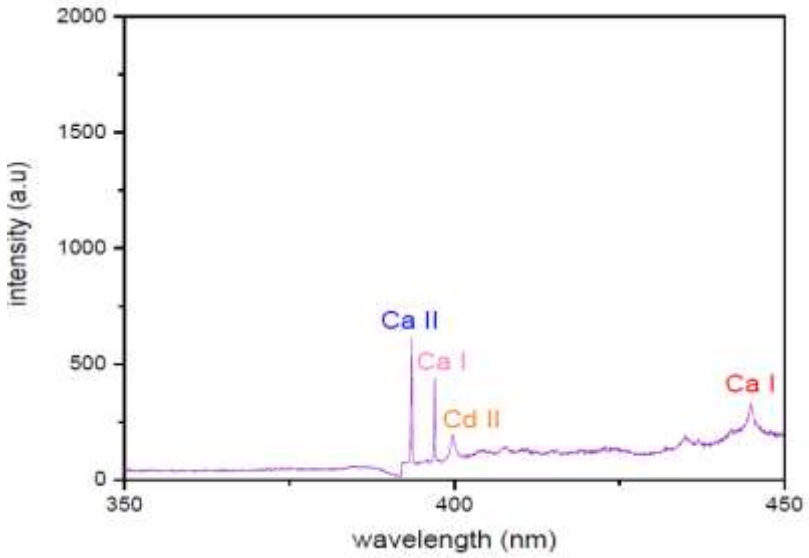


Figure 06: The LIBS spectrum extracted from plasma plume of doped material of the hairs sample in wavelength ranges (350-450 nm)

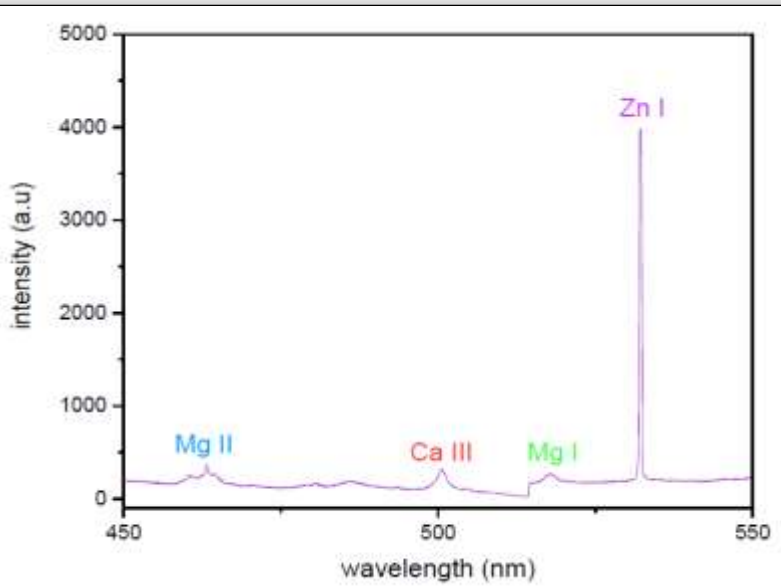


Figure 07: The LIBS spectrum extracted from plasma plume of hairs sample in wavelength ranges (450-550 nm)

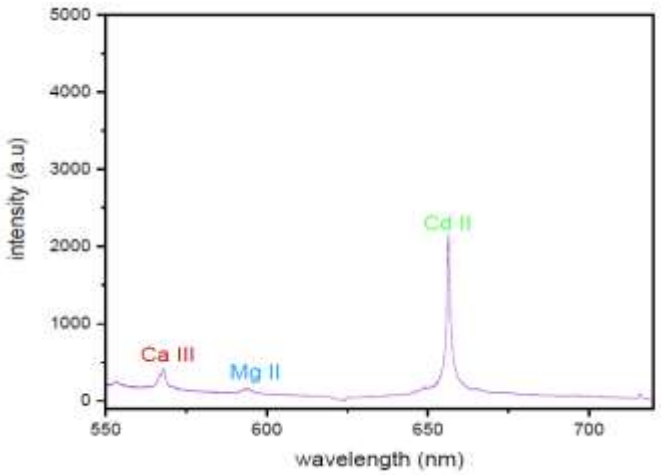


Figure 08: LIBS spectrum take out from the plasma plume of hairs sample in the wavelength ranges (550-720 nm)

3.2 Plasma Diagnostics

For CF-LIBS quantification, the plasma temperature (Te) and electron density (Ne) were determined. Boltzmann-plots of Mg and Ca lines (Figure 4.12) gave 3820 382 K (Mg neutral lines), 3690 ± 369 K (Mg ionized lines), 3740 ± 374 K (Ca neutral lines), 3580 358 K (Ca ionized lines) for the electron temperature Te nothing could be obtained.

The electron density, determined from the Stark broadening of Hα, was of the order of 10^{17} cm^{-3} and LS T was present as the McWhirter criteriou was satisfied.

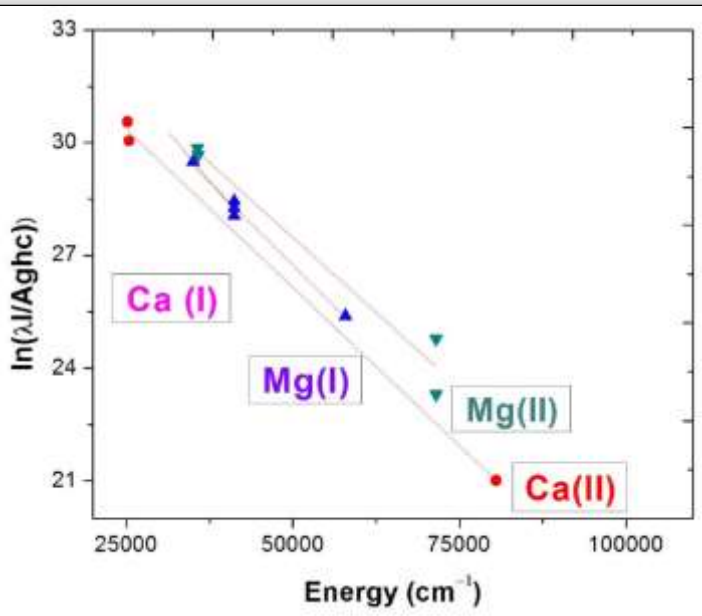


Figure 09: Boltzmann Plot of Ca and mg (neutral and ionized) Lines

3.3 Quantitative Analysis by CF-LIBS

The CF-LIBS quantification was carried out using the plasma conditions. The concentration profiles of all hair samples were: **Ca > Mg > Pb > Cd > Zn.** Samples from patients with cancer of the stomach had increased levels of Pb and Cd, and higher concentrations of Ca, Mg, and Zn were detected in the controls.

3.4 Comparison with AAS

CF-LIBS results were compared with AAS (Table 4.22). A representative sample showed:
Ca: 36.5% (CF-LIBS) vs. 39.4% (AAS)
Mg: 31.4% vs. 28.2%
Pb: 13.8% vs. 15.2%
Cd: 10.7% vs. 11.8%
Zn: 7.6% vs. 5.4%

Table 02: Metals and Heavy Metals observed in Hair sample-1

Element	CF-LIBS	AAS
Ca	36.5%	39.4 %
Mg	31.4%	28.2 %
Pb	13.8%	15.2%
Cd	10.7%	11.8%
Zn	7.6%	5.4%

The concentration levels of Pb, Zn and Cd in hairs samples is of our interest. In all hairs samples the concentration levels of the respective elements is initiate as Ca>Mg> Pb > Cd > Zn.

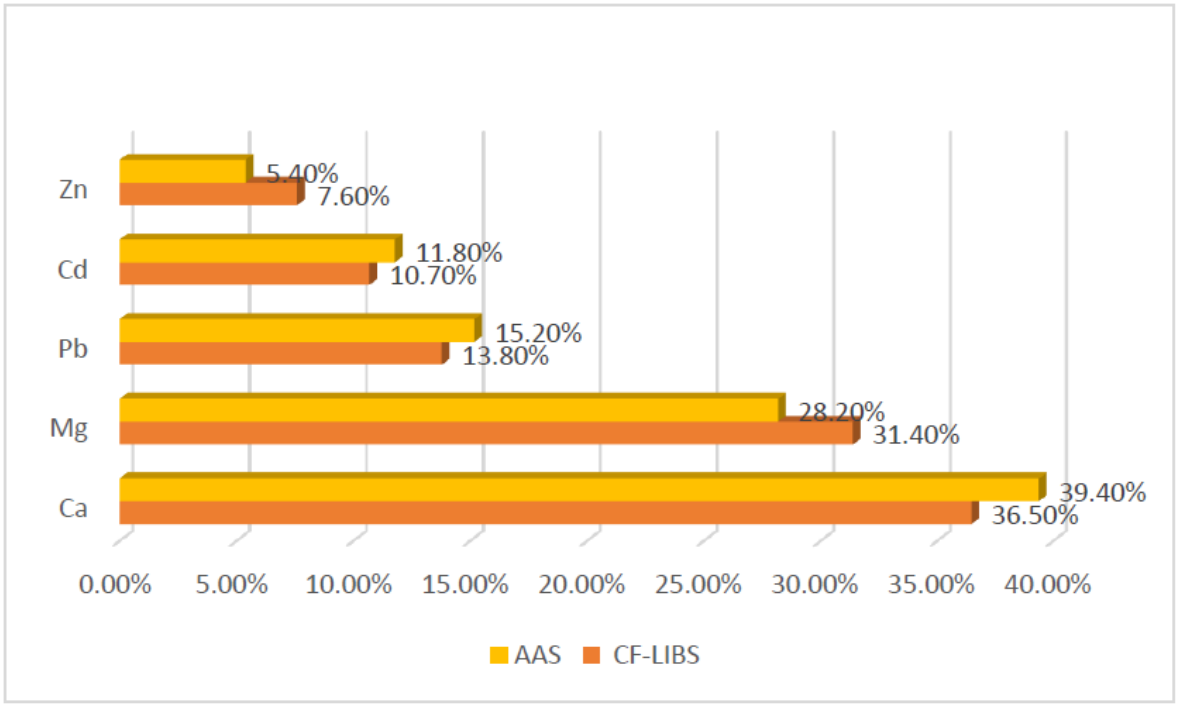


Figure 10: Comparison of CF- LIBS technique with AAS

A good correlation was observed between the two techniques ($R^2 > 0.95$ for Ca, Mg and Zn), justifying CF-LIB as a useful alternative. **Figure.10** depicts the AAS/CF-LIBs comparison.

3.5 Biomedical Relevance

The elevated levels of Pb and Cd and reduced levels of Ca, Mg, and Zn in cancerous tissue suggest the contribution of toxic metals in the causation of cancer and the perturbation of the balance of essential trace elements. The agreement between CF-LIBS and AAS confirms that LIBS could be used as a quick, screening, non-destructive tool for trace metal cancer biomarkers for the diagnosis of cancer.

Conclusion

This research proved that CF-LIBS is a suitable technique for element analysis of human hair samples for cancerous and non-cancerous subjects. The triple and single inter-lines confirmed that LTE conditions were satisfied so that the amount of elements was correctly determined. The levels of essential elements (Ca, Mg, Zn) were observed to decrease and those of toxic elements (Pb, Cd) were found to increase in cancerous hair as compared to non-cancerous hair, suggesting their possible involvement in carcinogenesis. Good agreement between CF-LIBS and Atomic Absorption Spectroscopy (AAS) proved the accuracy of LIBS as a quantitative technique. Generally, the results indicate that LIBS-based elemental analysis of hair could be a potential non-invasive method for detecting cancer markers.

Future Work

Further work should avoid the limitations on sample size to obtain higher-level of confidence in statistics, and expand to more trace elements, improve the sensitivity by advanced LIBS, e.g., double-pulse excitation. To move LIBS based hair analysis into the clinic, integration with complimentary spectroscopic techniques and the development of standardized procedures are necessary.

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