



## Research article

## Validity of laser induced breakdown spectroscopy (LIBS) in determining age and sex from tooth specimens

Priyanka<sup>a</sup>, Unnikrishnan V<sup>k</sup>, Prasanna Mitra<sup>c</sup>, Srikant N<sup>a,d</sup>, Joanna Baptist<sup>a,e</sup>, Keerthi K<sup>b</sup>, Ravikiran Ongole<sup>a,\*</sup><sup>a</sup> Department of Oral Medicine and Radiology, Manipal College of Dental Sciences Mangalore, Light House Hill Road, Mangalore, 575001, Manipal Academy of Higher Education, Karnataka, India<sup>b</sup> Department of Atomic and Molecular Physics, Manipal, Manipal Academy of Higher Education, Karnataka, India<sup>c</sup> Department of Community Medicine, Kasturba Medical College, Mangalore, Light House Hill Road, Mangalore – 575001, Manipal Academy of Higher Education, Karnataka, India<sup>d</sup> Department of Oral Pathology and Microbiology, Manipal College of Dental Sciences, Mangalore, Light House Hill Road, Mangalore – 575001, Manipal Academy of Higher Education, Karnataka, India<sup>e</sup> Department of Oral and Maxillofacial Surgery, Manipal College of Dental Sciences, Mangalore, Light House Hill Road, Mangalore – 575001, Manipal Academy of Higher Education, Karnataka, India

## ARTICLE INFO

## Keywords:

LIBS

Age estimation

Sex estimation

Tooth specimen

## ABSTRACT

**Background:** To determine age and sex from tooth specimens using Laser-Induced Breakdown Spectroscopy (LIBS). **Materials and methods:** A total of 170 human teeth samples between age of 15–86 years of both male and female sex were obtained for the study based on the inclusion and exclusion criteria. All the tooth samples were mounted and ground sections of about 1.5–2 mm thickness were prepared. Each tooth sample was subjected to LIBS along the cemento-enamel junction and spectrographs were acquired that denoted the elemental presence in each tooth sample.

**Results:** The elemental concentration of Ca I, Ca II, P and Fe in all the teeth samples declined as the age progressed. The Peak intensity of Ca, P and Fe was observed in the second decade of life. The overall concentration of Fe exhibited highest intensity in both male and female samples when compared with other elements assessed. The general concentration of all the elements were relatively higher in the female samples when compared to those in males.

**Conclusion:** The CEJ seems to be a better site for elemental analysis of tooth since multiple tooth sites are represented in this region and the other sites of teeth such as enamel or roots of teeth may tend to get fractured, and may not be available for the analysis. The signal intensities of trace elements seen to reduce with increase in age. However, certain external factors also seemed to have an influence on these outcomes.

## 1. Introduction

Identification of deceased individuals is important both for crime investigations and humanitarian reasons. As a part of forensic medicine, when human remains are found, two basic problems have to be resolved: 1) Identification of the individual and 2) The cause of death [1]. Soft tissues decompose and usually impairs the identification process. Comparatively, skeletal remains including teeth are more resilient to destruction and provide much needed information. The human dentition can be a good source for determining the age and sex based on morphology and employing various odontometric techniques. Occasionally, the teeth

obtained may not retain its morphological characteristics. It may then be useful to analyze the elemental composition of such samples to give us hints regarding the episodes of environmental contact and further to identify individuals [1].

The exact composition of the tooth may vary based on the site, sex and age. Enamel is a biomaterial the normal levels of calcium, phosphorous and other trace elements are subject to change and may also influenced by the dental diseases [2]. The chemical composition of the tooth has been studied extensively and it is believed to contain various inorganic substances such as Calcium, Phosphorous, Oxygen, Fluoride, Potassium, Sodium, Magnesium, Zinc, Iron, Strontium [3]. considering

\* Corresponding author.

E-mail address: [ravikiran.ongole@manipal.edu](mailto:ravikiran.ongole@manipal.edu) (R. Ongole).

the fact that human teeth can withstand high temperatures and decomposition, and usually the last of the tissues in the body to disintegrate. They form ideal specimens in the identification of an individual. Current methods employed in age and sex estimation are visual methods, morphometric measurements, radiocarbon dating of enamel, orthopantomography, etc.

Laser-induced breakdown spectroscopy (LIBS) has proven to be a reliable analytical tool that provides a chemical composition of any specimen (solid, liquid, or gas), both analytically and quantitatively. LIBS has been successfully used in analyzing compositions of biological samples obtained from humans such as tissues and bones [1, 4]. We believe that this ability of LIBS to detect elements, in tooth specimens can be harnessed in forensic medical applications. In this study, we used LIBS to assess the age and sex of tooth specimens using laser-induced breakdown spectroscopy (LIBS).

## 2. Material and methods

Ethical clearance was obtained from the Institutional Ethics Committee, Manipal College of Dental Sciences, Mangaluru (19095) for our study. All the teeth samples were obtained from the out patients who have undergone treatment, department of Oral and Maxillofacial Surgery, Manipal College of Dental Sciences, Mangalore. Informed consent was obtained from the patient to obtain their basic demographic data such as name, age, and sex. The examiner was blinded to the age and sex of the obtained tooth sample. Patients of 15 years of age and above were considered for the study. About 170 sound permanent teeth which were extracted for orthodontic purposes, impactions, and mobile teeth from different patients were used for this study. Teeth that were carious, fractured, and hypoplastic were not used for the study. Similarly, teeth that were previously restored were not considered for the study.

After extraction, each tooth sample was labelled and retained in a plastic container containing hydrogen peroxide which was used as a chemical disinfecting agent. Then, the disinfected tooth samples were mounted using a Plaster of Paris/Dental stone. The mounted tooth samples were sectioned using a cast trimmer until the thickness was about 1.5–2 mm. Elemental analysis of each tooth sample was done along the respective cementoenamel junction (CEJ) using LIBS was undertaken in the Department of Atomic and Molecular Physics, Manipal.

The LIBS setup for tooth analysis is shown in Figure 1 and the detector used was a Czerny Turner (Shamrock 193i) spectrometer coupled with intensified charge couple device (ICCD). An Nd: YAG laser with a wavelength ( $\lambda$ ) of 532 nm was used as radiation source. The pulsed laser beam of 6mm diameter with 6 ns pulse duration and 10 Hz repetition rate with an energy of 5mJ (before beam splitter) is focused on to the sample. After beam splitter, the energy was reduced to 2.5 mJ. A bi-convex lens with a focal length of 10 cm was employed to focus the laser beam on the tooth sample. The sample was secured on a mobile x-y-stage. Numerous lenses/mirrors are also used in the form of light collecting system.

## 3. Results

All the statistical analysis was done using SPSS version 20.0. The teeth samples were divided into seven age groups specifically from 11 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 81 to 90 as mentioned in Table 1. The youngest age from whom the tooth was obtained was from female (15 years) and the oldest age from whom the sample was obtained was from a man (86 years). The mean age of the teeth samples obtained was 45.323. Of the 170 samples, 82 samples were obtained from males and 88 from females. The majority of teeth were obtained from the age group of 51–60 of about 43 teeth samples, followed by 21–30 age group (42 tooth samples), 61 to 70 (32 tooth samples) and only one sample was obtained from the 81 to 90 age group.

Using LIBS, each tooth specimen was subjected to a minimum of 10 trials, of which 8 most significant trials were considered for analysis. Each trial run was compared with an existing database given by NIST [5, 6, 7]. LIBS intensity is proportional to the elemental presence (i.e., the concentration of trace elements in the tooth sample).

In our study, in order to maintain uniformity and reproducibility, the target area for laser pulse was fixed at the level of CEJ in each tooth sample. Ca emission lines were observed to be most significant at two wavelengths specifically at 422–423 nm (Ca I) and 429–430 nm (Ca II) [8]. P emission lines were most significant at 533–534 nm and Fe at 444–445 nm. During our initial trial runs using the tooth sample before initiating the study we also found the presence of lines for trace elements such as Zinc (Zn), Magnesium (Mg), Manganese (Mg), Fluorine (F), and Copper (Cu). Zn lines were found at the wavelength range of 411 nm, Mn at 518 nm and Mg at 550 nm, F at 527 nm, and Cu at 503 nm range

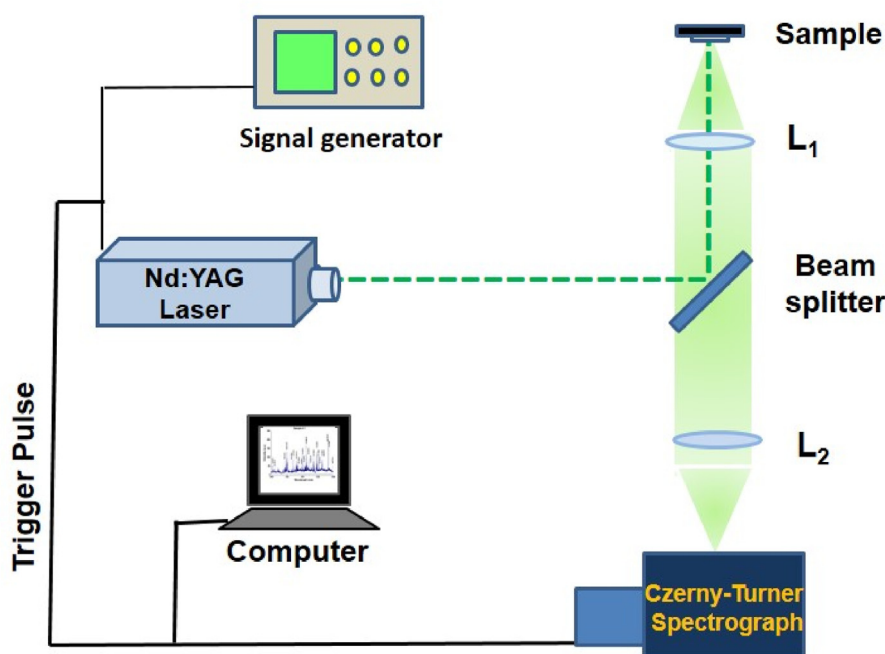


Figure 1. Schematic representation of the LIBS set-up [31].

**Table 1.** Comparison of trace elements in age.

Age group	No. teeth samples	Average intensities of the elements			
		Ca I	Ca II	P	Fe
11–20	10	12413.9	10557.6	2002.74	14794.7
21–30	42	11311.2	9152.22	1561.54	11585.1
31–40	19	9618.03	7812.47	1545.89	10076.6
41–50	15	8987	7035.56	1571.84	8805.65
51–60	43	8247.24	6128.3	1266.7	7762.51
61–70	32	7488.11	6039.84	1399.9	8391.91
71–80	8	5877.275	4324.925	1057.5	5683.6
81–90	1	5065.6	3572.2	883	4632.2
<b>Total samples</b>	<b>170</b>				

(Figure 2). Though these emissions were significant, we decided to focus our attention on the major elements, such as Calcium (Ca), phosphorus (P), and Iron (Fe). Hence, we decided to study Calcium (Ca), phosphorus (P), and Iron (Fe) lines in detail.

During the analysis of the 170 samples, the data points falling far from the fitted trend line were considered as outliers, approximately 16 results were outliers and these were included in the final analysis. The correlation of the trace elements (Ca I, Ca II, P and Fe) with the age groups showed statistically significant results ( $P < .001$ ) whereas on sex correlation with elemental composition of teeth samples the P value was not statistically significant. There was also significant negative correlation between elemental composition (Ca I, Ca II, P and Fe) of teeth samples and age groups (Table 2). On comparing the sex with elemental composition of teeth samples in male and females, it was observed that all the mean values of Ca I, Ca II, P and Fe in females is higher than males.

### 3.1. Ca, P and Fe in various age groups

On analysing the average intensities of Ca I, Ca II, P and Fe in all the age groups, it was commonly found that the highest intensity was observed in the second decade of life (11–20 age group) and least was seen in the 81 to 90 age group (Table 1). There was significant decrease

in the average intensities of all these elements with increase of age. However, few tooth samples showed a reverse trend which were not correlating with the other intensity values in the same age groups (Figures 3 and 4).

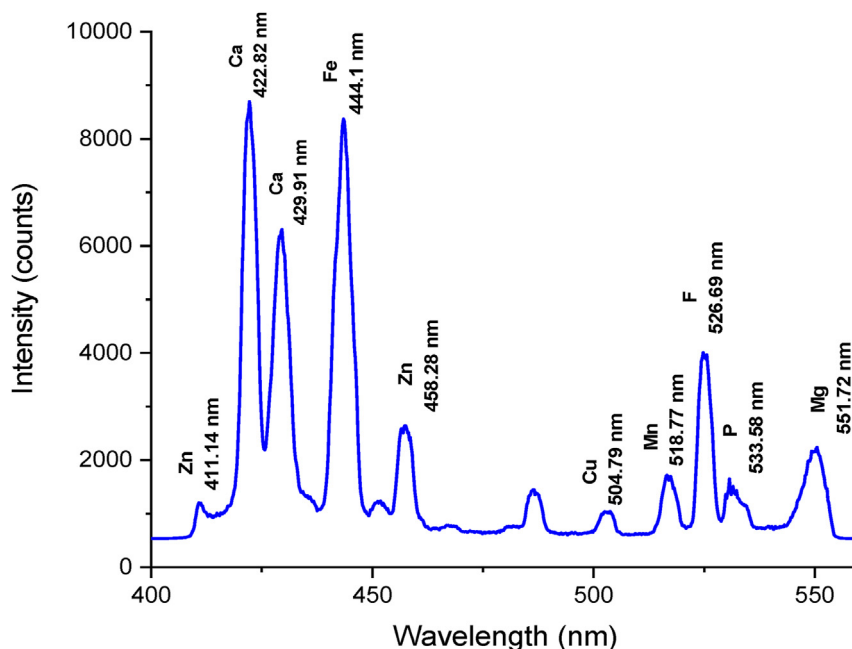
The predictability of age in female teeth samples was found to be as followed: Ca I ( $R = 43\%$ ), Ca II ( $R = 39\%$ ), P ( $R = 12\%$ ), and Fe ( $R = 37\%$ ). There was a moderate correlation between the age and female teeth samples. The predictability of age in male teeth samples was found to be as followed: Ca I ( $R = 26\%$ ), Ca II ( $R = 27\%$ ), P ( $R = 46\%$ ), and Fe ( $R = 16\%$ ). There was a weak correlation between the age and male teeth samples. We believe these results can be attributed to various environmental factors and underlying systemic conditions of the individuals which were not documented in this study [9, 10, 11].

### 3.2. Comparison of trace elements (Ca, P, and Fe) in sex

In both the female and male samples, it was found that the average intensity values of Ca I, Ca II, P and Fe dropped as the age increased with very weak correlation and P value being not significant. When we look at the intensities of Ca (I and II), P and Fe in various female age groups, we generally see that Fe exhibits the highest intensity followed by Ca I and Ca II. P has the least intensity (Figure 5).

It is also interesting to note that the concentration (intensity) of P in females generally remains stable at about 2500 counts. Also noteworthy is that these concentrations gradually tend to decline with increase in age. The concentration of all these elements (Ca, P and Fe) in female samples are at the highest in the second decade of life, remains stable in the third and fourth decade of life and starts to decline from the fifth decade onwards (Figure 5).

In teeth samples obtained from men, similar to female teeth samples, the concentration of Fe seems to be relatively higher when compared to other trace elements (Figure 6). The concentration of Ca I was more than Ca II and was higher in the second and third decade of life (13200 counts), that gradually decreased with increase in age (Table 1). It is also interesting to note that the concentration (intensity) of P in males generally remains stable at about 1600 counts in all the age groups. There was a considerable increase in concentration of all the elements (Ca I, Ca II, P and Fe) in the sixth decade of life (graph 5). Also, the overall



**Figure 2.** Various trace elements identified using the NIST database. All the characteristic elemental lines with their wavelengths are marked on the graph. Emission lines of Ca and Fe along the CEJ of tooth dominate the spectrum.

Table 2. Sex wise age correlation.

	S.no	Parameters being correlated	N	Correlation(r)	P value
Female	1	AGE & Ca I	88	-0.662	<0.001
	2	AGE & Ca II	88	-0.63	<0.001
	3	AGE & P	88	-0.356	0.001
	4	AGE & Fe	88	-0.615	<0.001
Male	5	AGE & Ca I	82	-0.51	<0.001
	6	AGE & Ca II	82	-0.525	<0.001
	7	AGE & P	82	-0.219	0.049
	8	AGE & Fe	82	-0.405	<0.001

concentration of all the elements in male samples were relatively lower when compared to those of the female teeth samples (Figures 3 and 4).

#### 4. Discussion

Age and sex determination of unknown individuals in forensic medicine helps in creation of the individual's profile, thereby aiding in the identification. When forensic personnel are faced with totally decomposed bodies or left with human skeletal remains the identification of age and sex is challenging [12, 13]. Over the last few decades, teeth have been utilized as excellent sources for determining age and sex. From the basic visual or clinical methods, morphometric measurements to radiological evaluation and advanced methods like PCR, enamel protein, amino acid racemization and radiocarbon dating of enamel have been used widely [4, 14, 15, 16].

From the Last decade and so, the human teeth have been drawing attention as a potential, biological, modelling sample. Ingestion of the trace elements by humans leads to accumulation in the hard tissues and can be influenced by environmental factors [17]. Studies have shown that there is no active metabolism of elements after formation of dentine. Also, the amount of trace elements may change with age. The presence and concentration of trace elements may provide useful information with regards to the age and sex [9].

In our study we employed laser-induced breakdown spectroscopy (LIBS) to assess the trace elemental composition of teeth in age and sex. LIBS is one of the most simple, rapid, and uncomplicated techniques for the evaluation of the concentration of elements [18, 19]. The core objective of LIBS is to perform a chemical analysis at the atomic level, possibly qualitatively (i.e., determining the existence of certain elements) or quantitatively (i.e., determining the relative concentration of distinct elements in a sample using the collected spectra). We believe that LIBS will help in the identification of the composition of teeth. Thereby, help in the age and sex estimation [20]. LIBS has been successfully applied in the field of dentistry to achieve various goals such as to investigate the elemental presence of sound teeth, carious teeth, dental restorative materials, and also to study the changes in the chemical composition of teeth.

In various studies, the elemental analysis of teeth was done at different sites for instance the surface of enamel, coronal portion of enamel and dentin, enamel-dentin junction, dentin-pulp margin, pulp, CEJ, root canal dentin, apical part of enamel [2, 5, 21, 22, 23, 24, 25, 26, 27]. The tooth can relatively withstand various environmental influences and is the last to decompose (mass disasters) [17]. Generally, it has been seen that the incisal edges, crowns and various parts of roots tend to fracture and may not be available for the study. In our study, we chose the CEJ as the site for analysis.

Considering that this was first of its kind study to differentiate male and female teeth samples, in our study, we obtained teeth from apparently healthy individuals. We used healthy adult teeth from either sex. Teeth that were carious, fractured, restored and hypoplastic was not utilized for the study. Other authors have used specimens that were carious and non-carious, where it was identified that the Ca/P concentration decreases in a caries affected teeth using LIBS. *Toscano DD* distinguished the elemental presence in the carious and non-carious enamel and dentin using LIBS [27]. *M. Saiki et al.* the elemental composition in sound and carious enamel-dentin tissues using instrumental neutron activation analysis [6]. *Zahran NF, et al.* reported that the mean concentrations of Na, Al, K, Cr, Mn, Co, Cu, Zn, Mo, Ag, Bi and U in the pulp of permanent teeth were lower in caries than healthy teeth pulps using Inductively coupled plasma mass spectrometry [29].

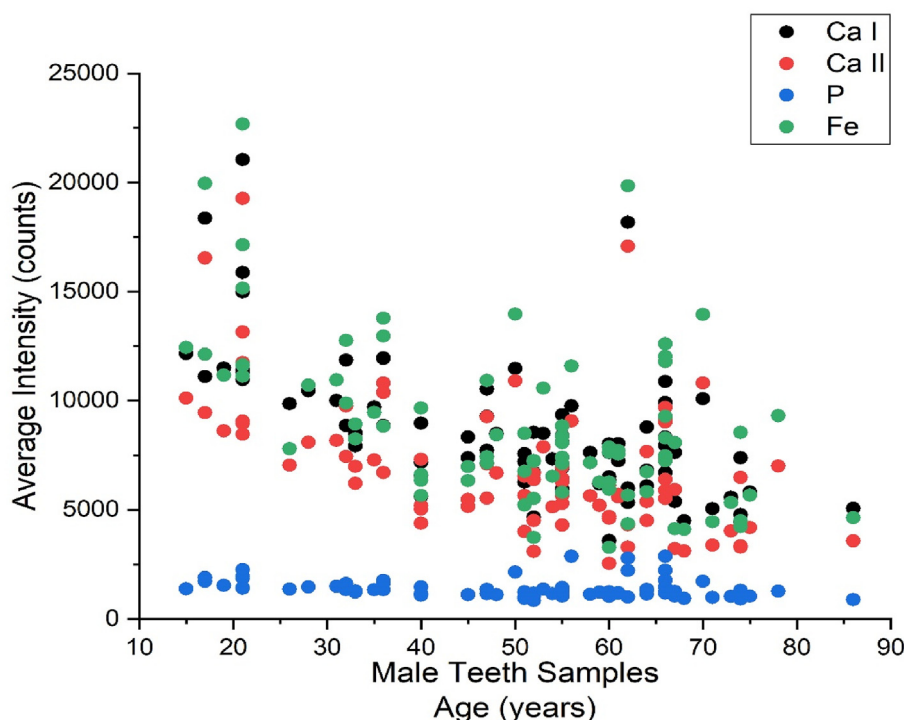


Figure 3. Comparison of Ca I, Ca II, P and Fe in all the age groups of male teeth samples.

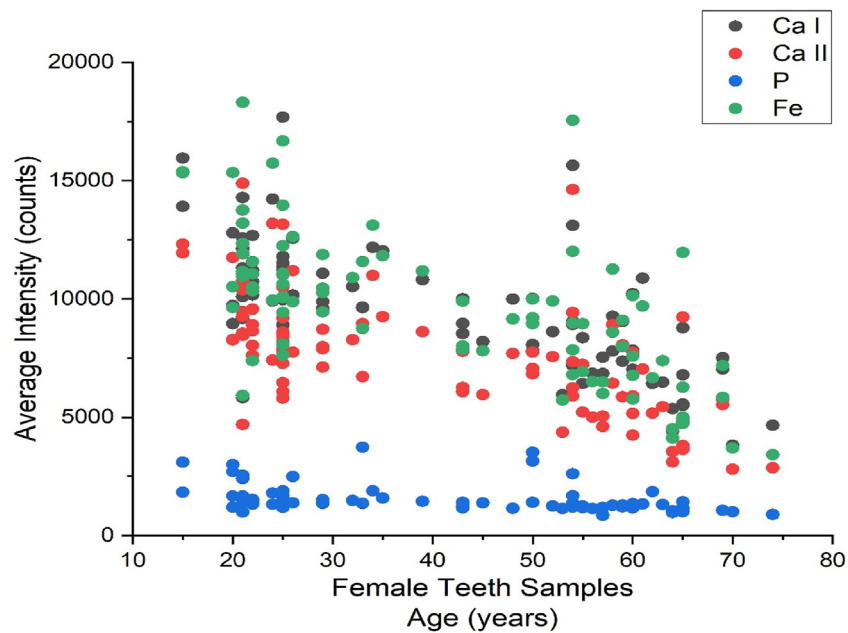


Figure 4. Comparison of Ca I, Ca II, P and Fe in all the age groups of male teeth samples.

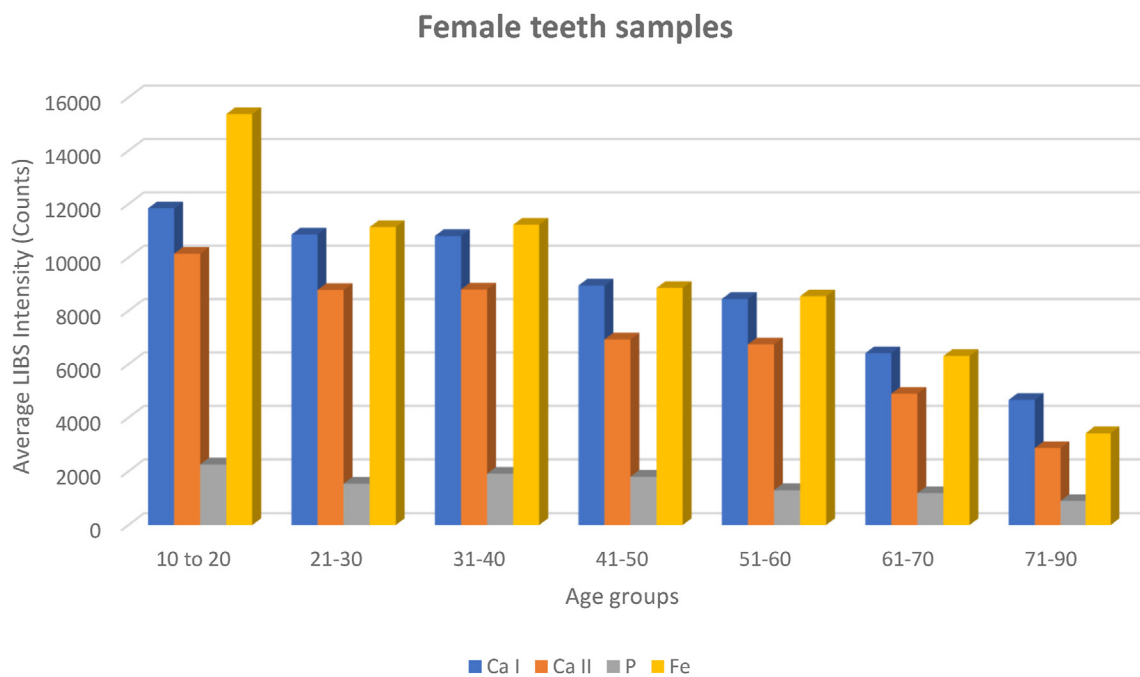


Figure 5. Comparison of Ca I, Ca II, P and Fe in all the age groups of female teeth samples.

In our study we chose to analyze Ca, P and Fe since these were the major elements. Also, other minor trace elements such as Zn, Cu, F, Mg and Mn were too noticed in the observed spectra of each tooth samples. In our study, we have identified the lines for Ca, P and Fe in the CEJ region of each tooth sample. Previous studies demonstrated that the Ca and P are the major elements in bones and teeth of humans and Fe along with other few elements (Al, Cu, Cd, F, Co, and Mn) as minor trace elements [21].

In our study, the teeth samples were categorized into seven age groups from 11 to 90 years of age comprising of 10 years difference in each group. In our study, we analyzed two Ca lines emitted in the spectra, which was identified at the wavelength of 422–423 nm (Ca I) and 429–430 nm (Ca II). Likewise previous studies were conducted using the

ratio of Ca I (422.67 nm) and Ca II (396.84 nm) in the characterization of apical enamel using LIBS [8].

Our study revealed that the concentration of Ca I and Ca II in the age group of 11–20 were high in both male and female tooth samples. Similarly, a literature revealed that the concentration of Ca decreased in the deciduous teeth of older children compared to younger children using atomic absorption spectroscopy [16, 21].

Our study revealed that the changes occurring in the chemical composition of teeth may be age-dependent progress. The concentration of Ca, P, and Fe exhibited changes in all the age groups relative to that of both males and females. The percentage of Ca, P, and Fe seen substantially decreased previously with increase in age which correlated with the studies conducted previously [10].

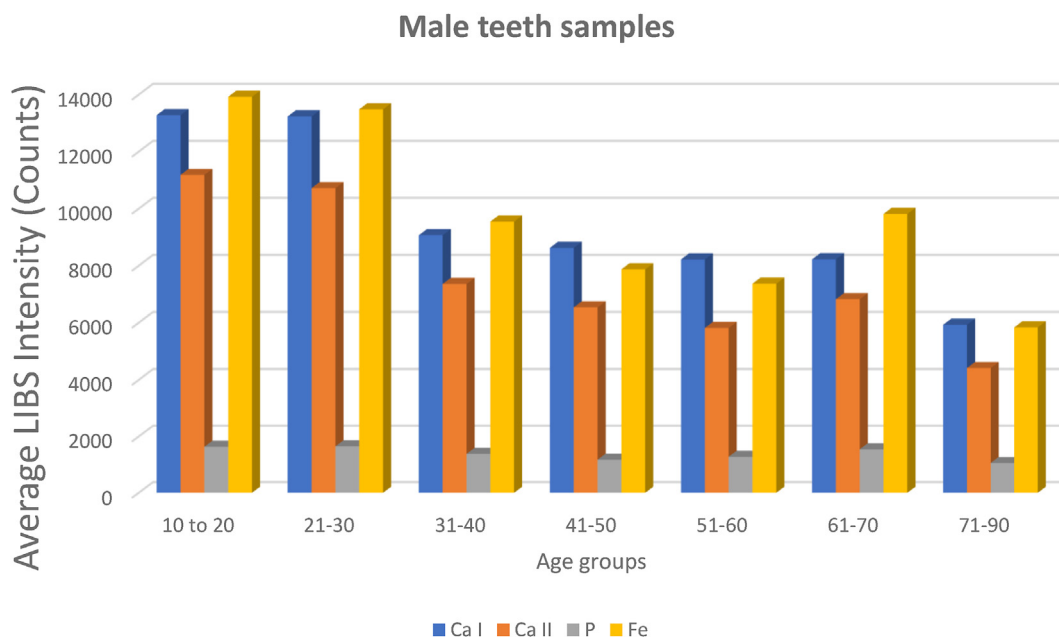


Figure 6. Comparison of Ca I, Ca II, P and Fe in all the age groups of male teeth samples.

Our study demonstrated that there was a significant decrease in the mass of Ca and P in both males and female samples from the third decade onwards. Also, there was a slight increase in the P and Fe values especially evident in the sixth decade of male teeth samples. The overall concentrations of Ca, P, and Fe seemed to be greater in females compared to males. All of these above findings were consistent with the previous studies [2].

Our study revealed that the Fe lines were greater in the male tooth samples and the Ca and P lines were seen more in the female samples. Likewise, the literature revealed that the iron content in males was more compared to females and the Ca/P content was subsequently high in females than the males by using the atomic emission spectrometry (AES) technique [30].

## 5. Conclusion

LIBS seems to be a promising tool in the identification of trace elements in tooth samples. The concentration (signal intensity) of these trace elements seem to had correlation with age and sex of an individual. The cemento-enamel junction (CEJ) seems to be a better site for elemental analysis of tooth since multiple sites (enamel, dentin and pulp chamber) are present in this region and the other sites of teeth such as enamel or roots of teeth may tend to get fractured. The signal intensities of trace elements seen to reduce with increase in age. However, certain external factors also seemed to have an influence on these outcomes. Female teeth samples seem to have higher trace elemental composition when compared to the males.

## 6. Limitations and recommendations

Our study to assess the validity of the Laser-Induced Breakdown Spectroscopy (LIBS) to determine age and sex from tooth specimens showed that LIBS seems to be a promising tool and should be considered as an adjunct to assess age and sex in forensic applications. However, we would like to highlight certain limitations in our study and like to propose certain recommendations for further studies. In our study, we did not compare the LIBS technique with any other existing gold standard techniques to confirm the results for age and sex application. We also did not assess the systemic condition of the patient, their environmental exposure and the concentration of trace elements in blood. Apart from

taking the above into consideration it may be interesting to study the significance of other trace elements present in the human teeth samples. We also propose that further studies can be considered including the teeth which are carious and have other inherent developmental disturbances. It may be useful to study differences between deciduous and adult dentition and also undertake studies in other geographical locations and ethnic groups.

## Declarations

### Author contribution statement

Dr. Priyanka: Performed the experiments; Analysed and interpreted the data; Wrote the paper.

Dr. Unnikrishnan VK: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Dr Ravikiran Ongole: Conceived and designed the experiments; Wrote the paper.

Dr. Prasanna Mitra, Dr. Srikant N and Dr. Joanna Baptist: Contributed reagents, materials, analysis tools or data.

Keerthi K: Performed the experiments.

### Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Data availability statement

Data will be made available on request.

### Declaration of interest's statement

The authors declare no conflict of interest.

### Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2022.e10946>.

## References

- [1] V.K. Singh, V. Kumar, J. Sharma, Importance of laser-induced breakdown spectroscopy for hard tissues (bone, teeth) and other calcified tissue materials, *Laser Med. Sci.* 30 (6) (2015 Aug) 1763–1778.
- [2] V. Zaichick, S. Zaichick, The effect of age and sex on calcium, phosphorus, and calcium-phosphorus ratio in the crowns of permanent teeth, *EC Dental Science* 5 (2) (2016) 1030–1046.
- [3] S. Kc, Development of human age and sex identification system from teeth, wrist and femur images, in: *Proceedings of International Conference on Sustainable Computing In Science, Technology And Management (SUSCOM)*, Amity University Rajasthan, Jaipur-India, 2019, February.
- [4] J. Kaiser, K. Novotný, M.Z. Martin, A. Hrdlická, R. Malina, M. Hartl, V. Adam, R. Kizek, Trace elemental analysis by laser-induced breakdown spectroscopy—biological applications, *Surf. Sci. Rep.* 67 (11-12) (2012 Dec 10) 233–243.
- [5] D. Hare, C. Austin, P. Doble, M. Arora, Elemental bio-imaging of trace elements in teeth using laser ablation-inductively coupled plasma-mass spectrometry, *J. Dent.* 39 (5) (2011 May 1) 397–403.
- [6] M. Saiki, L. Adachi, E. Adachi, Elemental comparison in sound and carious human teeth by instrumental neutron activation analysis, *J. Radioanal. Nucl. Chem.* 282 (1) (2009 Oct 1) 29–32.
- [7] Y. Ralchenko, NIST atomic spectra database, *MemorieDella Societa Astronomica Italiana Supplementi* 8 (2005) 96.
- [8] H. Suyanto, N.L. Trisnawati, I.K. Putra, I.E. Suprihatin, Characterization of human teeth by laser-induced breakdown spectroscopy, In *Journal of Physics: Conference Series* 1120 (2018), 012018. IOP Publishing.
- [9] M.S. Doğan, Relation of trace elements on dental health, *Trace Elements-Human Health and Environment* (2018 Sep 5) 71.
- [10] C.J. Brown, S.R. Chenery, B. Smith, C. Mason, A. Tomkins, G.J. Roberts, L. Sserunjogi, J.V. Tiberindwa, Environmental influences on the trace element content of teeth—implications for disease and nutritional status, *Arch. Oral Biol.* 49 (9) (2004 Sep 1) 705–717.
- [11] P.T. Bhattacharya, S.R. Misra, M. Hussain, Nutritional aspects of essential trace elements in oral health and disease: an extensive review, *Sci. Tech. Rep.* (2016 Jun 28) 2016.
- [12] E. Priya, Methods of skeletal age estimation used by forensic anthropologists in adults: a review, *Forensic Res Criminol Int J* 4 (2) (2017) 00104.
- [13] Schulz EM. Sex vs Sex in a Forensic Anthropological Analysis.
- [14] R.K. Srivastava, A. Kumar, I. Ali, P. Wadhvani, P. Awasthi, G. Parveen, Determination of age and sex and identification of deceased person by forensic procedures, *Universal Research Journal of Dentistry* 4 (3) (2014) 153–157.
- [15] C. Monali, P. Pritam, M. Tapan, D. Kajal, Sex determination: a view of forensic odontologist, *Indian J Forensic Med Pathol* 4 (2011 Oct) 147–151.
- [16] A. Fischer, D. Wiechula, C. Przybyla-Miszczela, Changes of concentrations of elements in deciduous teeth with age, *Biol. Trace Elem. Res.* 154 (3) (2013 Sep) 427–432.
- [17] A. Khalid, S. Bashir, M. Akram, A. Hayat, Laser-induced breakdown spectroscopy analysis of human deciduous teeth samples, *Laser Med. Sci.* 30 (9) (2015 Dec) 2233–2238.
- [18] Oprea C, Szalanski PJ, Gustova MV, Oprea IA, Buzguta VL. Trace element distribution in human teeth by X-ray fluorescence spectrometry and multivariate statistical analysis. arXiv preprint arXiv:1406.4387. 2014 Jun 14.
- [19] Iyengar GV, Tandon L. Minor and Trace Elements in Human Bones and Teeth.
- [20] R. Gaudio, N. Melikechi, Z.A. Abdel-Salam, M.A. Harith, V. Pallechi, V. Motto-Ros, B. Busser, Laser-induced breakdown spectroscopy for human and animal health: a review, *Spectrochim. Acta B Atom Spectrosc.* 152 (2019 Feb 1) 123–148.
- [21] N.D. Shashikiran, V.S. Reddy, M.C. Hiremath, Estimation of trace elements in sound and carious enamel of primary and permanent teeth by atomic absorption spectrophotometry: an in vitro study, *Indian J. Dent. Res.* 18 (4) (2007 Oct 1) 157.
- [22] V.K. Unnikrishnan, K.S. Choudhari, D. Kulkarni Su, R. Nayak, V.B. Kartha, C. Santhosh, B.M. Suri, Biomedical and environmental applications of laser-induced breakdown spectroscopy, *Pramana* 82 (2) (2014 Feb) 397–401.
- [23] R.M. Hassan, D.G. Mohamed, Cementoenamel junction in Egyptian maxillary first premolar: scanning electron microscopy and energy-dispersive X-ray analysis study, *Int. J. Adv. Res.* 3 (4) (2015) 545–556.
- [24] S.K. Eren, E. Uzunoglu, B. Sezer, Z. Yilmaz, İ.H. Boyaci, Mineral content analysis of root canal dentin using laser-induced breakdown spectroscopy, *Restorative dentistry & endodontics* 43 (1) (2018 Feb 1).
- [25] E. Klimusko, K. Orywal, T. Sierpinska, J. Sidun, M. Golebiewska, Evaluation of calcium and magnesium contents in tooth enamel without any pathological changes: in vitro preliminary study, *Odontology* 106 (4) (2018 Oct) 369–376.
- [26] A.C. Fernández-Escudero, I. Legaz, G. Prieto-Bonete, M. López-Nicolás, A. Maurandi-López, M.D. Pérez-Cárceles, Aging and trace elements in human coronal tooth dentine, *Sci. Rep.* 10 (1) (2020 Jun 19) 1–4.
- [27] El Tayeb S, Geith MZ, Harith MA. Human Enamel Analysis in Ancient Egyptians and Contemporary via LIBS.
- [29] K. Nganvongpanit, K. Buddhachatt, P. Piboon, T. Euppayo, P. Mahakkanukrauh, Variation in the elemental composition of human teeth and its application for feasible species identification, *Forensic Sci. Int.* 271 (2017 Feb 1) 33–42.
- [30] S.I. Soroko, I.A. Maximova, O.V. Protasova, Age and sex features of the content of macro and trace elements in the organisms of children from the European North, *Hum. Physiol.* 40 (6) (2014 Nov) 603–612.
- [31] K. Keerthi, S.D. George, R. Nayak, S. Chidangil, V.K. Unnikrishnan, Role of geometric configurations in optimizing the LIBS signal enhancement, *Optik* 251 (2022 Feb 1), 168387.