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Sensitivity and Specificity of Various Techniques for the Trace Elemental Analysis on Teeth: A Critical Review

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Abstract. Trace elements play an important and complex role in the human metabolism and, although present in only minute concentrations, can be either beneficial or detrimental to health. Qualitative and quantitative analyses of the trace elements from human teeth are performed using various methods. Atomic absorption spectroscopy (AAS) and Inductively coupled plasma atomic emission spectroscopy (or mass spectroscopy) (ICP-AES, MS) are the most popular methods for trace element analysis Previous studies have set the framework for developing methods aimed at elemental analysis from bones and teeth but there are areas in which the field still needs to progress. Research must be undertaken to expand the techniques to include more precision, non-destructiveness and accuracy and high detection limit, including additional human bones and teeth, non-human bones and teeth, non-skeletal materials, and to trace present and past life behaviours like migration pattern, nutrition status and occupational or environmental exposure, human provenance and reconstruction of migration pathways as well substance abuse in human skeletal remains and in living beings.

Keywords: Trace element analysis; Teeth; Bio monitoring; Environmental exposure.

1. Introduction

Teeth are the strongest and most mineralized part of the human body to destroy. An important characteristic of teeth is their durability. In cases of burning the jaws and teeth are well protected by the tongue, lips, and cheeks. They are also frequently relatively well preserved even after being buried for thousands of years[1–3]. For these

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reasons teeth are frequently used for the identification of dead bodies of unknown identity. Apart from natural disasters and traffic accidents, the need for dental identification also arises during and after wars. Teeth are the great recorders of environmental risk to toxic metals and of nutritional conditions[4-6]. Trace elements and their isotopes in bones and teeth are used in a variety of endeavours, including diet of past humans and other hominids, dating archaeological specimens, forensic discrimination of individuals and their origins and movement histories, conditions of cremation and contamination of cremated remains, differentiating between dental and osseous tissue from other materials, identification of specimen provenance and burial conditions and discriminating processes of post-mortem trace element uptake[7-10]. Carbon and nitrogen isotopes are used to reconstruct diet, while oxygen and strontium isotopes are used to determine geographic origin. Strontium and lead isotopes in teeth and bone can also be used to reconstruct migration in human populations and cultural affinity[11,12].

Trace elements can collocate in the calcified tissues of the body such as bone, teeth, and fingernails. Bio monitoring of toxic elements in calcified tissue has developed into paramount means to appraise an individual’s health status and environmental risks. The existence of toxic elements in the surroundings consequences their possibility to harm humans. Teeth are recently drawing attention for their potential as biological modelling investigation samples due to their easy extraction and slow substance metabolism. Concentration analysis of teeth has not been conducted to investigate the relationship between sample and environment only, but also for estimation of age and sex[13-16]. The investigations on the concentration of various chemical elements in teeth depending on age, the place of living, the environment, work conditions as well as diseases, pathological conditions, dietary habits and diseases causing dental caries have been carried out for a long time[8,17-30]. Teeth can be an important deposit of exogenous substances accumulated both in the pulp and in the calcified tissues; they are an invaluable source of data from a toxicological point of view also. In this way, elemental distribution in teeth can provide information about the physiology of elements, environmental influence, information about identity, ethnicity, geographic origin and toxicology of long term drug abuser, dietary habits, or contamination by metallic amalgams used as a restorative material.

Human tooth is a complex system of specialized tissues: enamel, dentin, cementum, and pulp. Each tooth is basically made up of two parts: the crown and the root. Enamel serves to protect the underlying tooth, the next layer of which is dentine. The bulk of the pulp is similar in composition to a connective tissue, containing various types of cells, collagen fibres, nerve trunks, lymphatic and blood vessels. Tooth and especially dental enamel reflect past metabolic, nutritional, and pollution events. Tooth enamel is the most mineralized tissue of human body. Its composition is 96 wt. % inorganic material and 4 wt. % organic material and water. In dentin, the inorganic material represents 70 wt. %.

The tooth is predominately composed of hydroxyapatite (Ca3 (PO4)5OH) crystals. Bio apatite crystal formations is under direct metabolic and physiological control of the organism, and thus trace metal signatures, their chemistry and concentrations within the bio apatite reflect the load and status of the metabolism of trace metals. Enamel is the hardest tissue in the human body, it has a special property: Its composition is fixed before tooth eruption, and this could provide a historic record of trace elements absorbed during the early development stage. Thus useful information can be obtained by the carries-formation process, a person's prenatal nutrition, and exposure to toxic metals[26-29]. And for archaeological research, dental analysis can provide information about the geographical environment, climate condition and even what kind of food the ancients ate in the past.

During the last two decades, the list of elements of interest for human health related studies has been constantly expanding. Together with essential elements and well recognized toxic metals (Al, As, B, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, Na, P, Pb, S, Se, Si, Sn, V, Zn) the following groups of the elements have become a focus of attention for environmental sciences:

- Elements that may cause acute toxicity (Ag, Bi, Sb, Tl, W, etc.)
- Elements used in implants (Ag, Cr, Ni, Pt, Si, Ti, Zr) as well as in pharmacology (Au, B, I, Pd, Pt, Ti, etc.)
- Precious metals (in Pt-Pd-Rh-containing catalytic converters and dental alloys in a Pd or Au base)
- Rare earth elements, increasingly used in electronics, as imaging agents or in tracer experiments
- Actinides (U, Th, Pu in nuclear fuel reprocessing)

Enamel, unlike other human tissues, has a special property: Its composition is fixed before tooth eruption, and this could provide a historic record of trace elements absorbed during the early development stage. Thus, useful information can be obtained by the caries-formation process, a person's prenatal nutrition, and exposure to toxic metals. Various studies had proposed the classification of microelements in relation to caries potential and designated them as cariostatic (F, P); slightly cariostatic (Mo, V, Cu, Sr, B, Li, Au); without influence on caries (Ba, Al, Ni, Fe, Pd, Ti); supporting caries (Se, Mg, Cd, Pb, Pt, Si), and uncertain (Br, Be, Co, Mn, Sn, Zn, and I)[29]. Qualitative and quantitative analyses of the trace elements from human teeth are performed using various methods. Atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectroscopy (or mass spectroscopy) (ICP-AES, MS) are the most popular methods for trace element analysis. These methods have high sensitivity (ppm—ppb); however, they require a liquid specimen. Therefore, solid specimens should be solubilised, for example, with an acid treatment.

Objectives of this review study are:

- To have an overview on various techniques of trace elemental analysis done on teeth.
- To give outline their qualitative and quantitative nature and their roles in bio monitoring.
- To determine strengths and limitations of various techniques on trace elemental analysis on teeth.

2. Different techniques of trace elemental analysis

2.1 Trace elemental analysis using an AAS (atomic absorption spectrophotometry)

A technique used to identify the presence and concentration of substances by analyzing the spectrum produced when a substance is vaporized and absorbs certain frequencies of light. AAS is used particularly for detecting the concentrations of metal ions in solutions, dried and powdered teeth digested with concentrated nitric acid and analyzed by the atomic absorption spectroscopy (AAS) method. In the past decades, this method was widely used, though it is destructive in nature it can detect following elements: Pb, Mn, Fe, Cd, Cu, Ni, Co, Zn and Cr by AAS, and Na, Ca and K by flame photometer. Downside of this technique is that it requires relatively large sample quantities (1-3 ml) and intended for metallic/metalloid atomic species, not non-metals or intact molecular species or refractory elements. In the feasibility study of estimation of trace element analysis on large population, some of the potentially cariostatic elements are determined by flame and electro thermal vaporization AAS (FAAS and ETV-AAS). They offer a possibility of reconstructing diet in ancient teeth samples and estimating biological profiling specially age and sex[16].

2.2 Trace elemental analysis using an ICP (Inductively Coupled Plasma)

Inductively Coupled Plasma Techniques can be very powerful tools for detecting and analyzing trace and ultra-trace elements. Over the past years, ICP-MS has become the technique of choice in many analytical laboratories for providing the accurate and precise measurements needed for today’s demanding applications and for providing required lower limits of detection. Thirty-four elements (Si, Al, Fe, Ca, Mg, K,Mn, Ti, P, Li, Be, B, V, Cr, Co, Ni, Cu, Zn, As, Sr, Y, Nb, Mo, Ag, Cd, Sn, Sb, Ba, La, Ce, W, Pb, Bi, and Zr) were analyzed by inductively coupled plasma–atomic emission spectrophotometry technique, while fluorine was analyzed by ion sensitive electrode method. Inductively coupled plasma atomic emission spectrometry (ICP-AES) appears to be the most commonly used method of determining the concentrations of these trace metals in teeth, but ICP mass spectrometry, ICP-OES have also been used to determine the concentrations of trace metals in human and animal teeth, as well as bone. As this technique have excellent sample throughput, very wide analytical range of detection and its applications for isotope abundance ratio measurements in various forensic samples relevant to monitoring occupational or environmental exposure, human provenance and reconstruction of migration pathways as well as toxicology research in drug cases. Some study used ICP (Inductively Coupled Plasma) technique to report the relationship between element levels to sex, age and implications to caries[13,14].Only disadvantages are its damaging in nature to sample and expensive operating cost.

2.3 Trace elemental analysis using LA–ICP–MS (Laser Ablation - Inductively Coupled Plasma – Mass Spectrometry)

LA–ICP–MS has recently emerged as a method to solve questions that may be answered by an examination of the spatial distribution of trace elements in hard tissues including human teeth and bones[15,17]. Trace element profiling in biological samples, using laser ablation as a sample presentation method, may provide vital information for environmental monitoring. Teeth can serve as monitors of environmental pollution because heavy metals accumulate in the mineral phase of the dental tissues during their formation[15,31]. High sensitivity, low detection limits, along with depth profiling and surface area scanning makes LA–ICP–MS an attractive technique for analysis of the spatial distribution of elements[31-37]. The technique requires little sample preparation and handling and therefore reduces the risk of sample contamination. Moreover, repeated analysis can be performed as only microgram quantities of sample are consumed in a single laser shot. An advantage of this approach to tooth analysis is that it is relatively non-destructive to the sample unlike atomic absorption spectrometry, ICP-atomic emission spectrometry or ICP-MS where time dependent information is destroyed in the sample preparation process. Hence a single sample can be studied repeatedly, used for inter-laboratory comparison studies, or archived for later use. LA-ICP-MS is superior to scanning electron microscopy with X-ray fluorescence (SEM-XRF) analysis in sensitivity. Although SEM-XRF can locate the neonatal line and illustrate the surface morphology the XRF technique is insufficiently sensitive to detect the trace levels of elements incorporated into the matrix. This technique is very valuable in studies of the intra-inter human discrimination of skeletal remains found in the articulated or clustered *remains in same place,* palaeodietary patterns and intoxication by occupational or environmental exposure related studies[15,31-37].

2.4 Trace elemental analysis using LIBS (Laser Induced Breakdown Spectroscopy)

A fast and minimally destructive method based on Laser Induced Breakdown Spectroscopy (LIBS) or Laser Micro Analysis (LMA) has been developed and applied to the classification and discrimination of human bones and teeth fragments. The methodology can be useful in Disaster Victim Identification (DVI) tasks. In recent years, Laser Induced Breakdown Spectroscopy (LIBS) has become a powerful analytical tool because of its ability to carry out a rapid qualitative and quantitative analysis of different samples, able to provide real time spectral fingerprint of the elemental composition of the sample[38-42]. Given its speed, high throughput, minimal destructive to sample, microanalysis and ease of use. Only very little sample preparation is needed. Detection limits for solid samples are usually in the range of a few tens of parts per million with little or no sample preparation. It is a simple, low-cost, and non-destructive technique. It is possible to perform quantitative LIBS analysis of trace element concentrations in calcified tissue [38-39]. It is possible to distinguish unequivocally between healthy and caries infected teeth. Hence, LIBS analysis could be implemented and used in dental drilling using lasers, a technique which increasingly is being tested in dental laboratories and is reaching maturity. It is reported to diagnose the seawater drowning in an advanced stage of putrefaction by LIBS[43]. Many studies have been reported the use of LIBS techniques in forensic study such as accumulation of potentially toxic elements in calcified tissue and evaluating diet or mobility from changes in the Sr/Ca concentration ratio of the enamel[44-47]. Other applications may well emerge in which the capability for spatially resolved quantitative analysis is required.

2.5 Trace elemental analysis using NAA (Neutron Activation Analysis)

The method is simple, fast, multi-elemental, and non-destructive. NAA constitutes an advantageous method for dental tissue analysis as it enables simultaneous multi element determinations and does not require sample dissolution. NAA can detect up to 74 elements depending on the experimental procedure, with minimum detection limits ranging from 10^{-7} to 10^{-15} g/g, depending on the elements and matrix materials. Milligram-sample analysis; where samples are precious or limited especially in ancient teeth samples. Also, because there is no chemical pre-separation, the sensitivity of the method is dependent upon the sample matrix.NAA is an efficient technique for the determination of many important chemical elements in tooth tissues such as dentine and enamel. By means of NAA, the age dynamics, palaeodiet reconstruction, physiopathological phenomena can be studied in teeth[48-49].

2.6 Trace elemental analysis using PIXE (Proton Induced X-ray Emission spectroscopy)

Proton induced X-ray emission spectroscopy (PIXE) has been widely used for trace element analysis. This technique is multi-element, non-destructive and, for thick targets, has the advantage of requiring very little sample preparation. The PIXE technique offers the advantage of analysis, without the necessity for time consuming digestion, thereby minimizing the potential for error resulting from sample preparation, offers better peak to noise ratios and consequently much higher trace element sensitivities. Because of its inherent characteristics, PIXE offers a great potential for trace element analysis in teeth. The detection limits obtained by using 2MeV proton and 3MeV helium ion beams are of the same order, with some improvements for $Z<29$. Detection limit on the order of 1-10ppm is obtained[50-51].For quantifying purposes, the PIXE method, being an absolute method, is very reliable. Hence this non-destructive external PIXE technique for the elemental analysis of materials is perfectly suited for the trace elemental analysis of teeth for species identification and dental caries of the human being[50-53].

2.7 Trace elemental analysis using XRF (X-Ray Fluorescence spectroscopy)

XRF combines highest accuracy and precision with simple and fast sample preparation for the analysis of elements from Beryllium (Be) to Uranium (U) in the concentration range from 100% down to the sub-ppm-level. Unsuitable for analysis of very light elements e.g. H to Ne The µXRF technique in combination with synchrotron radiation has proved to be a useful and rapid method for multi-elemental analysis in this kind of samples. With a simple sample treatment, several dental tissues, dental calculus, and the interfaces between them could be analyzed. In addition, this technique gives insight into the mineralization degree of different areas of calculus. The intrinsic characteristics of synchrotron radiation permit to implement spectrochemical analysis with spatial resolution on the micrometer scale, high efficiency for trace elements determination and short time of analysis requirement. µSRXRF technique is a useful tool for the study of elemental distribution in biological samples such as human teeth[54-56].Elemental profile by XRF can serve as a tool to differentiate human from non-human samples (species identification) , as well as it is possible to distinguish dental restorative resin brand which serve as unique identification in forensic investigations[54-64].

3. Discussion

Trace elements (TE) play important role in all bio-medical systems. They take part in all metabolic processes, being components of different enzymes catalyzing chemical interactions in living cells. TE deficiency or excess with respect to the human physiological level has been found in patients with certain diseases, including cancer. The study of the role of the essential trace elements has been emphasized in the search for the possible causes[65]. For medical purposes very often blood and soft tissues are analyzed in order to diagnose a disease. Teeth are recently drawing attention for their potential as biological modelling investigation samples due to their easy extraction and slow substance metabolism. Geographical diversity of trace element concentrations may be genetically determined by quantitative distinctions in element mass involved in human metabolism. In this case, trace element content is a consequence of population adaptation in relation to definite environment factors (climatic, geographical, geochemical, etc.) and is necessary for the maintenance of normal vital functioning of the human organism. However, numerous investigations have proven that trace element content in teeth depends on the geochemical environment and nutrition[7,11,12,15,23]. Biological monitoring has been found to be a useful means of assessing body burden in humans. Human teeth provide useful information on the occupational and environmental exposure to trace elements. Trace elements are determined in man to assess the deficiency or excess levels of elements, which are related to health[8,23,26,32,54,65-67]. A variety of clinical samples are used for the analysis of the extent of deficiency or high level of elements. In assessing the environmental risks to toxic metals, where the exposure is infrequent and highly variable, the metal contents in biological tissues can provide a better estimate of the long term risk to the general public. In view of the advantages of teeth for monitoring body burden, it is convenient to survey exposure levels in exposed and unexposed population groups.

Various analytical methods have been applied for the determination of trace elements in enamel and they are listed in Table 1. They include atomic absorption spectrophotometry (AAS)[16-29] and emission spectroscopy (AES), proton induced X-ray emission (PIXE)[50-53], inductively coupled plasma- mass spectrometry (ICP-MS)[13,14], and laser ablation ICP-MS (LA-ICP-MS)[15,17,31-37]. Another potential possibility is to use low sample consumption, micro flow nebulizer with ICP-MS, which would reduce the sample volume and may improve element spatial resolution information in successive enamel layers. Although LA micro sampling together with the sensitive multi element capability of ICP-MS provides unique trace metal distribution profiles in human enamel and dentine layers, the concentrations reported are semi-quantitative. However, LA-ICP-MS has the potential to generate valuable quantitative elemental distribution information in teeth provided appropriate calibration strategies are developed[66,67]. Current methods for the spatial analysis of teeth using element-specific detection include synchrotron micro probe X-ray fluorescence (SXRF)[68], particle-induced X-ray emission (PIXE)[69]. However, these methods are expensive, which prohibits their use in large epidemiologic studies. Teeth have also been acid digested and analyzed by atomic absorption spectroscopy (AAS)[23], inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS)[70,71].Additionally, the lower spatial resolution compared to PIXE (0.1–10 mm) is better suited to imaging the entire tooth. LA-ICP-MS applications have been limited to spot ablation and small area rastering to determine an approximate spatial distribution of trace elements in teeth[31], but having great application in toxicological and archaeological sciences[32-37]. The various techniques detection limit is listed in Figure 1. The limitations of analytical inaccuracy and sample contamination are the source of error. Accuracy in the analysis can be overcome by using properly graded instruments, avoiding operator bias, ambient temperature, and pressure. The method should also include standard reference materials to avoid errors during analysis.

Table 1. Techniques for trace element analysis.

Technique	Principle	Strengths	Limitations
Atomic absorption Spectrometry (AAS)	Absorption of radiant energy, produced by a special radiation source, by atoms in their electronic ground state.	• Very easy-to-use • Widely accepted • Relatively inexpensive	• Low sensitivity • Single-element analytical capability • Cannot be left unattended (flammable gas)
Inductively Coupled plasma With atomic Emission Spectrometry (ICP-AES)	Measures the optical Emission from excited Atoms	• Best overall multi-element atomic spectroscopy technique • Excellent sample throughput • Very wide analytical range • Good documentation • Available for applications • May be left unattended • Easy-to-use	• Higher initial investment • Destructive analysis. • Spectral interferences (many emission lines) • Cost and operating expense.

		• Widely used • Isotope determination	
Inductively Coupled plasma With mass Spectrometry (ICP-MS)	Argon plasma used as Ion source; used for separating Ions based on their Mass-to charge ratio.	• Exceptional multi-element capabilities • Ability to perform isotopic analyses • Well-documented • Interferences and compensation methods • Rapidly growing application information • Detection limits equal to or better than GFAA with much higher productivity • May be left unattended	• Highest initial investment. • Method development more difficult than other techniques. • Destructive analysis
X-ray fluorescence (XRF)	-X-rays –primary Excitation source; -elements emit Secondary X-rays of a Characteristic Wavelength	• Non destructive analysis; • Relatively simple, cheap and quick analyses. • Accurate analyses of a range of elements. • Minimal sample preparation. • Good precision and accuracy. • Applicable over a wide range of concentrations.	• Cannot distinguish between different isotopes of an element. • Unsuitable for analysis of very light elements e.g. H to Ne. • Inter element (MATRIX) effects may be substantial and require computer correction.
Neutron activation Analysis (NAA)	-Conversion of stable Nuclei of atoms into Radioactive ones; -measurement of the Characteristic nuclear Radiation emitted by the Radioactive nuclei	• Most elements can be determined. • Non-destructive analysis • Highly sensitive procedure • No chemical pre-treatment • No sample loss	• Cannot be performed “in house” by industry. • The time required for the analysis. • Cannot provide information on some of the light elements.

		• No contamination	
Laser-induced breakdown spectroscopy (LIBS)	-A pulsed laser is used as the excitation source. -leading to the excitation of the material constituents and their spontaneous emission of element-specific radiation.	• No sample preparation necessary. • A fast and minimally destructive method • High-speed identification. • Depth-profiling of layered structures • Remote, non-invasive analysis • Compositional analysis of complex shaped objects. • Low instrumental cost • Easy to operate.	• Increased cost and system complexity. • Difficulty in obtaining suitable standards (semi-quantitative). • Large interference effects (including matrix interference and, in the case of LIBS in aerosols, the potential interference of particle size). • Detection limits are generally not as good as established solution techniques. • Poor precision - typically 5-10%, depending on the sample homogeneity, sample matrix, and excitation properties of the laser. • The possibility of ocular damage by the high-energy laser pulses.
Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)	The laser beam focused on the sample surface to generate fine particles a process known as Laser Ablation. -The ablated particles are then transported to the secondary excitation source of	• Can perform an ultra-highly sensitive chemical analysis. • No sample preparation. • Fastest analysis speed of all analytical techniques with the limit of detection approaching ppb level. • Depth profiling	• Knowledge of external standards used. • High instrumental cost. • The complexity of operation.

	the ICP-MS	• Elemental/isotope mapping • Excellent discrimination power, sensitivity, precision, and accuracy.	
Particle Induced X-ray Emission (PIXE)	X-rays produced following the excitation of target atoms induced by an energetic incident ion beam of protons or alpha particles.	• Multi-elemental analysis. • High sensitivity with good accuracy and precision. • Non-destructive analysis. • Minimal sample preparation. • Low cost per analysis.	• Requires well-equipped accelerator facility. • Interelement interferences in complex sample limit sensitivity lower the accuracy and precision. • By matrix effect thick targets difficult to estimate.

Human teeth fragments can be useful evidence when found in crime scenes and/or mass burials sites. The elemental and isotopic composition of these samples serve a information about environmental or occupational exposure events, past migration patterns , geographic origin, reconstructing diet and could also be used to distinguish from non-human teeth samples or from different individuals.

The development and application of robust analytical methods for the quantification of trace elements in these biological matrices may lead to a better understanding of the potential utility of these measurements in forensic analyses.

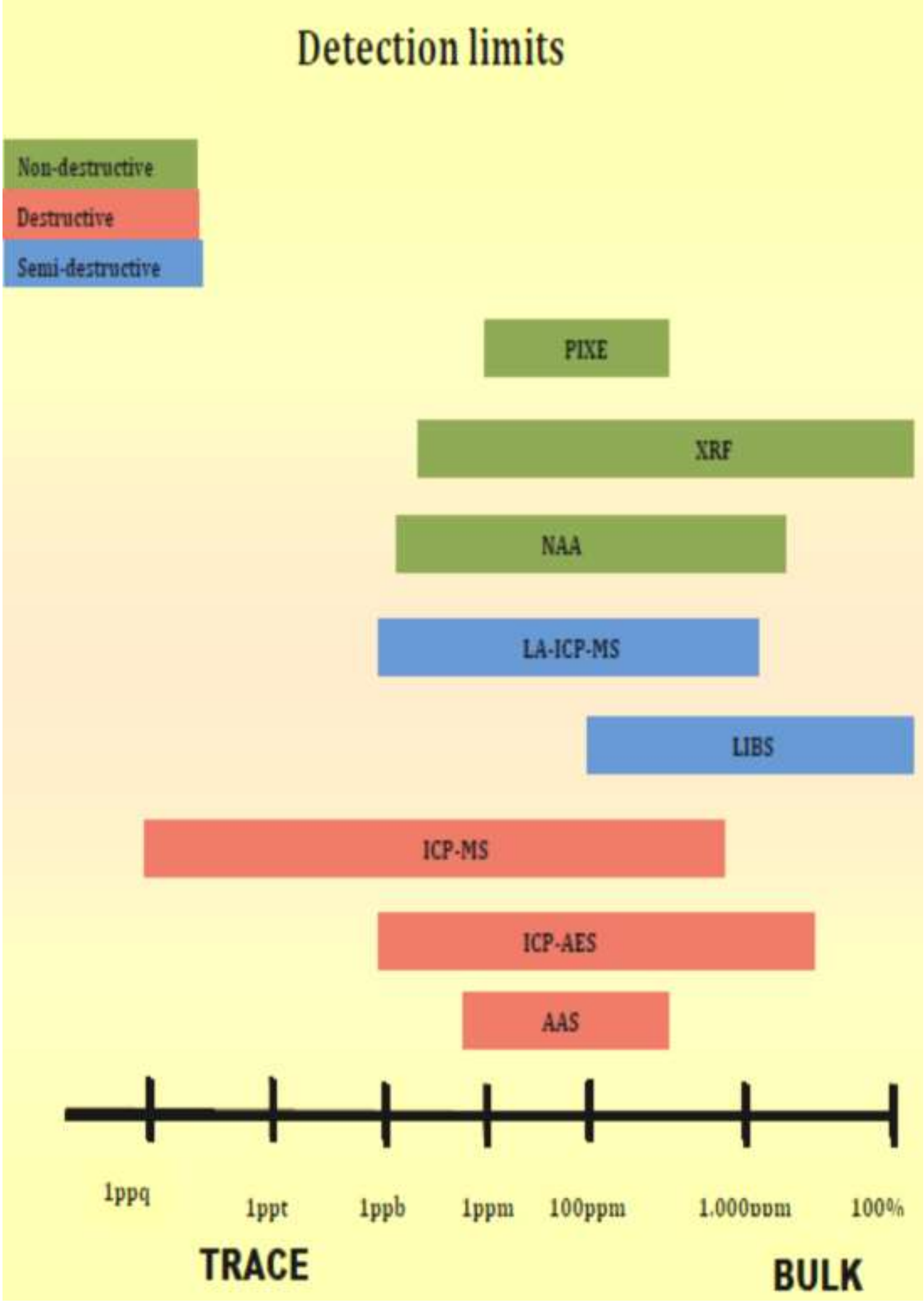

Figure 1. Detection limits of various techniques.

4. Conclusion

In conclusion, one can see that the destructive methods especially AAS (Atomic absorption spectroscopy) is not a highly useful technique for the analysis of trace elemental analysis from teeth. Nevertheless, it has to be considered as a valuable supplementary parameter given the scarcity of other available techniques. This method has important limitations, it does not provide information about the spatial localization of trace elements, neither the chance to follow a possible evolution of traces over time, and it is destructive, thus it cannot be used in unique pieces. Laser ablation methods are mainly used for the determination of trace elements with spatial resolution in the micrometer range in case, complex and expensive laboratory instruments are required. Laser Induced Breakdown Spectroscopy (LIBS) offers important advantages over these methods. It is an appealing technique compared with many other types of elemental analysis because setting up an apparatus to perform a LIBS measurement is very simple. LIBS require no previous sample preparation; it has no limitations in sample dimensions and shapes. The analysis is a micro destructive and low quantity of sample is needed with minimal damage. Chemical imaging of surfaces as spatial distribution profiles and depth profiling allows mapping and layer by layer analysis characterization of the composition of surface and substrates as well. Finally, the possibility of in situ and real time analysis that provides LIBS is an important plus for archaeological teeth. Each technique has its own advantages and disadvantage; it is clear from this review what niche every technique occupies in the field of trace elemental analysis. No technique is the answer to every analytical problem, and the forensic analyst must be rational in his or her selection of technique in respect of application.

Previous studies have set the framework for developing methods aimed at elemental analysis from bones and teeth but there are areas in which the field still needs to progress. Research must be undertaken to expand the techniques to include more precision, non-destructiveness and accuracy and high detection limit, including additional human bones and teeth, non-human bones and teeth. Additional research also needs to be contributed to differentiation of human and non-human skeletal materials. Subsequently, attention needs to be directed towards incorporating successful chemical differentiation methods into criminal investigations.

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Conflict of interest

There is no conflict of interest regarding publication of this article.

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