

Trend in the analysis of heavy metals in human teeth dentine: a review

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ABSTRACT

During the last years, many papers have been published on the analysis of heavy metals in human teeth. Nowadays, heavy metal toxicity is a common problem encountered in toxicology field. It has been found that heavy metal toxicity to humans is due to the long-term exposure to the heavy metals commonly found in traces, in our daily diet, drinking water and in environment as pollutants. Also, this exposure can occur through industrial effluents, coal and oil, and numerous consumer products, such as cosmetics. Teeth generally considered as an exo-skeleton part, accumulate heavy metals with high affinity and reflect information of long-term exposure. Only selected areas of dentine are enough for analysis instead of whole tooth. In the present paper, we have compiled the work done on the analysis of heavy metals in human teeth using different instrumental techniques.

Key Words: Heavy metals, Metal toxicity, Industrial pollution

Introduction:

In lifetime, humans have two sets of teeth. One set is called deciduous/temporary/milk teeth. These are 20 in number . 4 incisors, 2 canines and 4 molars i.e. 10 teeth in each jaw which begin to erupt at the age of six months and shed off starts at the age of

six years. Then the replacement of these teeth occurs by the permanent teeth which is the second set of teeth. These are 32 in number . 4 incisors, 2 canines, 4 premolars and 6 molars i.e. 16 teeth in

each jaw. Calcification of root of these permanent teeth is completed till the age of 22-25 yrs. The tooth is divided into mainly two parts: crown and root part. Crown is projected into the mouth and it is visible part and root is into the jaw, descends below the gum line. Main components of teeth are enamel, dentin, pulp, cementum and periodontal ligament. Enamel is the outermost and hardest part made up of rock-hard mineral and calcium phosphate. Dentin is hard tissue underlying the enamel containing microscopic tubes. Sensitivity or pain is caused when enamel damages as cold or heat enters the tooth through this path. Pulp living, softer, innermost central part of tooth. Nerves, connective tissue and blood vessels run through pulp. Cementum is the connective tissue layer that firmly binds the roots to the jawline and gums. Periodontal ligament is the tissue that helps to cushion the tooth tightly against the jaw. Tooth is surrounded by bony and mucosal structures. Dentin, enamel and cementum are three mineralized components of tooth presented in figure 1. (Vij, 2011; The Teeth (Human Anatomy) www.webmd.com). Since ages, humans are being exposed to the higher levels of heavy metals through both terrestrial and aquatic environment. Heavy metal are the metals with high atomic numbers, atomic weights, and densities.

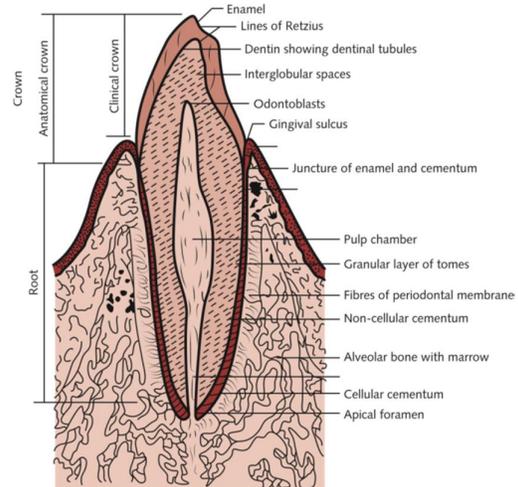


Figure 1 Human tooth section (Vij, 2011).

Some of these metals are essential for human body like calcium, magnesium, zinc, iron and some proved to be highly toxic for human being such as cadmium, arsenic lead, nickel, chromium, copper and mercury. Environmental factors like 1) anthropometric activities such as the utilization of coal, oil, gases, industrial effluents, urbanization, sewage, vehicular emissions, 2) agriculture sector which includes the use of fertilizers, insecticides, pesticides, rodenticides and herbicides are the major source of heavy metal exposure (Asaduzzaman et al., 2017). Heavy metals enter the human body through food chain via ingestion, inhalation and dermal contact. Once entered heavy metals accumulate in various organs including tissues, bones and teeth, affecting human health due to the chronic toxicity. Toxic metals are slowly absorbed into the body, but once get absorbed do not excrete easily and irreversibly accumulates into the body (Martin and Griswold, 2009). Due to the chemical co-ordination of heavy

metals and their oxidation-reduction properties, they help to control mechanisms in the human body such as homeostasis, transport and binding to cell constituents. By replacing original metals from their natural bindings, they bind with the protein sites causing malfunctioning of cells and finally it results into high toxicity. Different organs have different levels of accumulation of heavy metals. Amount of heavy metal accumulation into the body depends upon their binding to carrier molecules. Metallothionein (small proteins) plays a major role in the storage of heavy metals as it accounts for the unique metal binding properties. They can cause hepatotoxicity by binding with cysteine rich protein such as metallothionein and forms cysteine-metallothionein complex in the liver (Jaishankaret *al.*, 2014; Wan *et al.*, 2015).

During the development stage of calcified tissues, heavy metals get incorporated in them when exposed to heavy metals in their mineral phase. Urine and blood reveals information about recent exposures. While hair and fingernails can reflect information on exposure times from few months to years but get contaminated by dyes, shampoo, nail paints, airborne dust etc. Calcified tissues (bones and teeth) accumulate heavy metals with high affinity and reflect information of long-term exposure. Generally, bones are available for sampling however, teeth give a permanent record of recent and/or past exposure to heavy metals. Dental tissues

are very hard, made up of similar material as bones and generally considered as an exo-skeleton part. Analysis of heavy metals from human teeth has been used for classification of individuals in terms of heavy metal absorption and exposure as human teeth are biological tissue which is readily accessible. It doesn't require the whole tooth for study, selected areas of dentine are enough. Human dentine tissues do not undergo any mineral phase turnover and are part of exoskeleton. It offers several advantages over other bio-indicators thus, making it reliable indicator for environmental exposure to toxic heavy metals. As enamel and cementum surrounds the dentine so it has no effect of oral environment. In pulp, odontoblasts are situated that produce dentine continuously throughout the tooth lifespan until shed. Teeth hard tissues (dentine and enamel) are more beneficial over soft tissues such as kidney and liver, as are not subject to turnover once heavy metals are incorporated. As metals can cross the placental barrier therefore, teeth also include the heavy metal absorbed by mother at the time of pregnancy because their formation begins in the prenatal stage. Shed deciduous teeth can be obtained from schools, by approaching homes or from dental clinics. Permanent teeth are collected from dental clinics where these are generally extracted for orthodontal or any other reasons. Collection of ancient teeth is done from burial sites. Teeth can be stored well for

long time, only prevention is that they should not be in an acid or leaching environment. Therefore, calcified tissues to teeth retain various heavy elements to which they are exposed to and provide an accurate historical record. Estimation of age, sex, race, social status, occupation and habits can be done from teeth, so its analysis is also used for identification. To obtain reliable results or data from analysis, it is necessary to put considerable attention and care. (Argasinska *et al.*, 2004; Asaduzzaman *et al.*, 2017; Fergusson *et al.*, 1987; Shapiro *et al.*, 1973).

There are many instrumental methods used for the analysis of heavy metals from teeth such as Anode Stripping Voltammetry (ASV), X-Ray Fluorescence (XRF), Proton Induced X-Ray Emission (PIXE), Ion Induced X-Ray Emission (IIXE), Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma (ICP), Mass Spectrometry methods, Laser Induced Breakdown Spectroscopy (LIBS) (Fergusson *et al.*, 1987; Wan *et al.*, 2015). The aim of the present study is to review the current trend in the analysis of heavy metals from the tooth's hard tissue dentine.

Instrumental methods of analysis

Several studies have been conducted to detect the heavy metals in the human teeth using different chemical analysis techniques and analytical methods. The majority of the research has been

conducted using the discussed analytical methods.

1.1 Atomic absorption Spectrometry (AAS)

It is the most common technique used for the detection of trace elements in different samples especially in teeth samples. Nakamura *et al.*, 1995 developed the method of Graphite Furnace Atomic Absorption Spectrometry (GFAAS) with atomic atomization technique for the detection of trace amounts of Cadmium and lead in human and artificial teeth. Good agreement was obtained between the values obtained by GFAAS for dissolved samples and those which were directly introduced. 3.1-12% relative standard deviation was obtained for Cd and 0.9-8.1% for Pb. Nowak *et al.*, 1998 analyzed the biological (hair and teeth) and environmental (soil and air) samples collected from Zwardon with the help of AAS technique. The results show that the highest level of Pb was found in the age group of 61-80. It was found that changes in metal ratios for different age groups are certainly dependent on the metal homeostasis in human organisms. Tvinnereim *et al.*, 1999 detected zinc concentration in the 2747 primary teeth samples collected from Norway with the help of flame atomic absorption spectrophotometry. The results showed increase of 5.2% in the zinc concentrations. Tvinnereim *et al.*, 2000 analyzed 1200 Norwegian primary teeth samples for the presence of lead, zinc,

cadmium and mercury content with the help of Atomic absorption spectrophotometry. Statistically significant differences for lead, mercury and zinc were observed in carious and non carious teeth. Higher lead and zinc concentrations were found in teeth with roots than teeth without roots. Nowak and Chmielnicka, 2000 evaluated environmental exposure to Pb, Cd, Fe, Zn, Cu, Mn, Ni, Cr, Ca, Na and K in the inhabitants of Katowice District, on the basis of concentration in hair, teeth and nails. 785 tooth samples were analyzed using atomic absorption spectroscopy and increased accumulation of metals like Ni, Cr and Mn was observed in the teeth samples of the inhabitants. Malaraet *al.*, 2006 conducted a research to ascertain that the toxic elements do not differ significantly between the carious and non-carious teeth. 344 permanent teeth from residents of Poland were analyzed using AAS and no statistically significant differences between the concentration of these metals in the roots of non carious and carious teeth was found. Alomaryet *al.*, 2006 measured the concentrations of Pb and Cd in teeth samples of inhabitants of Jordan using AAS. The mean concentrations of Pb and Cd were 28.91 g/g and 0.44 g/g, respectively. The concentrations of these metals were higher in smokers than those from non-smokers. Al-Jubouri and Bashbosh, 2012 detected the cadmium levels in blood, hair, saliva and teeth samples of Iraqi workers using AAS. Increased cadmium

concentration was observed among the exposed subject. Fischer *et al.*, 2013 studied the changes of concentration of some elements (Mn, Fe, Mg, Cu, K, Cr, Pb, Cd and Ca) in deciduous teeth using AAS. Statistically significant decrease in the concentration was observed in teeth of older children as compared to younger ones. Fischer and Wiechula, 2016 studied the age dependent changes in the human teeth using AAS. The annual increase in the concentration of Pb in tissues of the teeth is approx 0.1 µg/g. Olovicet *al.*, 2020 determine the content of 12 metals in 23 teeth samples from two cities in Bosnia and Herzegovina using Flame atomic absorption spectroscopy (FAAS). High contents of Ca, Na, Mg, K, Cu, Zn and Fe were found in samples analyzed. Differences on the basis of gender, geographic location and smoking were more prominent between intra groups than for inter groups.

Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

Grobler *et al.*, 2000 studied the relation between lead concentrations in dental tissues and in blood using GFAAS. Tsuji *et al.*, 2001 detected the elevated lead levels in the teeth collected from inhabitants of remote western James Bay region of northern Ontario using GFAAS. Hernandez-Guerrero *et al.*, 2004 analyzed the teeth samples collected from people of Mexico to discover the lead concentrations using GFAAS. Highest lead concentration

was found in the 10-13 age groups. Baez *et al.*, 2004 detected lead and cadmium concentrations in the deciduous teeth samples of children living in Mexico using GFAAS. Statistical differences were only observed for cadmium according to age. Gomes *et al.*, 2004 detected the lead amount from the superficial enamel of teeth of 4-5 year old children using GFAAS. Higher lead concentration in teeth samples from the industrial area children was found than those living far from industries. Barton, 2010 analyzed 300 samples of 6-year children for detection of lead and cadmium in deciduous teeth, scalp hair, capillary blood using GFAAS. A positive correlation was found in between the Pb concentration in teeth and blood. Olympio *et al.*, 2010 conducted a study to determine the risk factors associated with the high lead levels in dental enamel of 160 adolescents using GFAAS. Zhang *et al.*, 2011 developed a new method based on dispersive liquid-liquid microextraction preconcentration and GFAAS for the determination of Cd and Pb in human teeth. Detection limit for cadmium and lead was found to be 5.6 and 45 ng/L.

1.2 Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

It is one of the most preferred techniques for the elemental analysis since it can provide excellent sensitivity, precision and accuracy in the analysis. Lot of studies had been conducted by various researchers using this technique.

Grunke *et al.*, 1996 determined the traces of heavy metals (Mn, Cu, Zn, Cd, Pb) in microsamples of teeth material using Inductively Coupled Plasma Mass Spectrometry with Electrothermal Vaporization (ETV-ICP-MS). Cox *et al.*, 1996 performed the profiling of dental tissues using laser ablation-inductively coupled plasma-mass spectrometry. It was found that mercury and gold content decreases from outer part of tooth to inner side whereas lead content increases from outer part to inner part. Al-Haddad *et al.*, 1999 conducted a study to determine the levels of cadmium, copper and iron from the shed deciduous teeth collected from children using Inductively coupled plasma spectrometer. Mean concentrations of Cd, Fe and Cu were found to be 53.8 ng/g, 20.22 µg/g and 2.78 µg/g. Franck *et al.*, 1999 detected the lead levels in deciduous teeth as the measure of internal exposure and low-level chronic exposure. It was found that lead concentration depends on the part and the type of tooth and number of risk factors including smoking and individual environment. Webb *et al.*, 2005 utilized inductively coupled plasma-atomic emission (ICP-AES) and mass spectrometry (ICP-MS) to determine lead, zinc and strontium concentration in deciduous teeth. Arruda-Neto *et al.*, 2009 utilized ICP-MS to reveal the increased lead concentrations in the teeth of inhabitants living near Guarapiranga dam above the internationally accepted limits. 40% higher lead concentrations were

found in the teeth of children living near dam than from the ones living in control region. Amr, 2011 analyzed the children's primary teeth and permanent teeth using ICP-MS to compare the content of trace elements. It was found that permanent teeth contained higher concentration of Na, Mg, Al, Fe, Ni, Cu, Sr, Cd, Ba, Pb and U, and significantly less Mn, Co, As, Se, Mo and Bi than the children teeth. Asaduzzaman *et al.*, 2017 analyzed human teeth dentine using ICP-MS to obtain metal exposure due to environmental pollution. It was found that higher metal levels were found in Chinese teeth as compared to Indian and Malay teeth. Fernandez-Escudero *et al.*, 2020 investigated the concentration of 25 trace elements in 150 human coronal dentine using ICP-MS and Atomic Emission Spectroscopy. Concentration of both toxic and essential elements was detected and increase in concentration of toxic (Pb, Li, Sn) and essential (B, Ba, K, Sr, S and Mg) was observed in coronal dentine related to age of the teeth but regardless of sex.

1.3 Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)

When there is need to determine the variation of the elemental content in the sample, then this particular technique of laser ablation-inductively coupled plasma-mass spectrometry is useful. Many authors have employed this technique in their studies to determine the

content of heavy metals in human teeth samples. Cox *et al.*, 1996 performed the profiling of dental tissues using laser ablation-inductively coupled plasma-mass spectrometry. It was found that mercury and gold content decreases from outer part of tooth to inner side whereas lead content increases from outer part to inner part. Kang *et al.*, 2004 studied the distribution of trace metals in deciduous tooth using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). The distribution of elements in the tooth was found to be dependent on individual's nutritional status and anthropogenic trace metal exposure. Castro *et al.*, 2010 conducted quantitative analysis on bone and teeth samples using sector field based inductively coupled plasma mass spectrometry coupled with laser ablation system (LA-ICP-SF-MS). Teeth samples from 14 different individuals were considered and it was found that separation of individuals was improved when elemental composition of enamel and dentine, cementum layers were used. Abdullah *et al.*, 2011 examined relative concentrations of lead, mercury and manganese in enamel regions of deciduous teeth from children with Autism spectrum disorders and high levels of disruptive behavior using LA-ICP-MS. No significant differences in levels of neurotoxicants were observed for children with ASDs (Autism spectrum disorder) and HDB (High levels of disruptive behavior). Hare *et al.*, 2011 performed the elemental

bio-imaging of trace elements in teeth using LA-ICP-MS. It was found that concentrations of Pb, Zn and Cd were higher in dentine and in the regions adjacent to the pulp. Hanc *et al.*, 2013 used LA-ICP-MS to evaluate the elements migration in human teeth with and without fillings. It was found that elements like Al, Ba, La, Sr gets migrated from fillings placed in dental cavities to healthy part of teeth. Guedeet *al.*, 2017 analyzed 23 tooth enamel and dentine samples from Muslim population in Tauste (North Spain) using LA-ICP-MS for the detection of trace elements and to investigate the diet patterns of Medieval Muslims. Results showed that high Pb content was found in some individuals due to intoxication by occupational exposure to anthropogenic lead. The chemical composition showed variation according to sex and age and it is directly related to food intake. Horton *et al.*, 2018 determined the associations between dentine biomarkers of Mn, Zn, Pb and later childhood behavior using LA-ICP-MS. It was reported that prenatal dentine Mn may be protective but excessive postnatal Mn increases the risk for adverse behaviors. Also, higher concentrations of Mn, Zn and Pb have an adverse impact on behavior.

1.4 X-ray Fluorescence (XRF)

XRF provides useful elemental information about the examined specimens without causing damage. Carvalho *et al.*, 2000 utilized X-ray

fluorescence spectrometry to analyze several teeth and pieces of bone originating from subjects of chalcolithic period. Higher concentration of Mn, Fe and Cu was determined in different tooth regions when compared to data from contemporary subjects and lead was found in order of magnitude of detection limit which is 1-2 µg/g. Baranowskaet *al.*, 2004 analyzed 38 teeth samples using XRF technique and metals detected were Ca, P, Mg, Al, S, K, Fe, Ni, Cu, Zn, Sr and Pb. Differences in content of Al, Pb and Fe were observed in women teeth and men teeth and high concentrations of Zn and Pb in teeth samples. Opreaet *al.*, 2009 analyzed several tooth samples using XRF to detect some trace element concentrations. Different elements were detected by using different light sources. Dias *etal.*, 2015 evaluated the differences in lead distribution in different bone and tooth structures using micro X-ray fluorescence. Also antemortem and postmortem Pb accumulation was also assessed. Highest amount of lead was found in the pulp and root of the tooth structure. Terueletal., 2015 compared the chemical composition of human teeth with other mammal species using Wavelength dispersive X-ray fluorescence (WDXRF). It was found that human and bovine enamel and dentine species showed the greatest similarity among the other species analyzed. Guerra *et al.*, 2016 determined the distribution of toxic elements in teeth treated with amalgam using energy

dispersive X-ray fluorescence (EDXRF). Higher concentration values of Hg were reported in the teeth treated with amalgam fillings. Nganvongpanit *et al.*, 2017 proposed new technique handheld X-ray fluorescence (HHXRF) for determining whether suspected tooth is authentic human tooth or not. It was found that human teeth can be effectively identified from other species but sex cannot be determined from the teeth samples.

1.5 Laser Induced breakdown Spectroscopy (LIBS)

Laser Induced breakdown spectroscopy is a versatile technique that is used to identify the elements present in the sample analyzed. It helps to identify metals as well as non metals in less than a second with minimal sample preparation, no waste generation and detect exogenous and endogenous substance elements in humans as well as animals. Sameket *et al.*, 2001 conducted quantitative detection of trace elements in samples (teeth and bones) using LIBS. Elements like Al, Pb and Sr was successfully quantified and identified. Alvira *et al.*, 2011 developed new method to determine Sr/Ca changes in the enamel of human lower third molar. Using femtosecond Laser Induced Breakdown spectroscopy (fs-LIBS), relative amount of strontium was determined. Alhasmiet *et al.*, 2015 determined the levels of toxic elements such as lead, cadmium and arsenic present in roots of extracted teeth

of smokers and non-smokers using LIBS. The respective elemental concentration (Pb, Cd, and As) 23.29, 0.26, 0.31, and 0.64. 11 ppm was found for non-smokers, 35.55, 0.33, 0.51, and 0.91. 1.5 ppm for smokers, and lastly 0.17, 0.31, 0.01, 0.05, and 0.05-0.09 ppm for control group. Khalid *et al.*, 2015 analyzed human deciduous teeth samples using LIBS. It was detected that elemental concentration of Ca, Fe, Sr, Zn and Pb was found to be highest in enamel, then in dentine and lowest in cementum. Suyanto *et al.*, 2018 characterized human teeth by LIBS. It was found that Indonesian human teeth contain some elements such as Ca, F, Si, Zn, Na, Sn, Ar, Li, K, Ce, Fe, Mn, Ti, Al, Cr and P.

1.6 Particle Induced X-Ray Emission (PIXE)

Particle Induced X-Ray Emission (PIXE) is a rapid and non destructive technique that is known for simultaneous multielement analysis with across the board sensitivity in the parts-per-million (ppm) range. Rizzutto *et al.*, 2006 done the teeth characterization using PIXE. The measurements of concentrations of Ca, P, O and C were done above the limit of 100µg/g. Rautray *et al.*, 2007 conducted the analysis of human bone and teeth using PIXE. Elements like P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Sr and Pb were quantified in the samples. Oprea *et al.*, 2007 utilized PIXE to measure the elemental content in human teeth. Detection of Ca, Cr, Cu, Fe

and Zn was done above the detection limits. Oprea *et al.*, 2009 characterized the chemical composition of human teeth using PIXE. The approach enabled the separation between the essential mineral teeth components and the pollutants deposited in teeth tissues. Rao *et al.*, 2010 estimated the trace elements in various parts of human teeth using external beam PIXE. In the study, elements including P, Ca, V, Mn, Fe, Ni, Cu, Zn, Ba, As, Sr and Pb were estimated on different parts of human teeth. Rautray *et al.*, 2010 in the study analyzed the elemental profiles of enamel, cementum and caries of human teeth using external PIXE. Ten elements were estimated and respective concentration of elements namely P, Ca, Fe, Zn and Pb in enamel is more than those in cementum.

1.7 Electrochemical methods

The electrochemical methods are often used to detect the metal ions present in the sample. These methods work on the principle of controlled voltage or current; potentiometry stripping voltammetry and they helps in analysis of transition metals and metalloids. Shapiro *et al.*, 1973 detected the lead levels in the dentine and circumpulpal dentine of deciduous teeth

using Anodic Stripping Voltammetry method. Tooth lead levels were compared and 2-3 times less lead was found in the teeth of Icelandic children as compared to normal American children. Bayet *et al.*, 2001 utilized differential pulse anodic stripping voltammetry after microwave oven digestion for the simultaneous determination of Cd and Pb in the deciduous teeth of children. Detection limits for Cd and Pb was found to be 0.078 and 0.323 µg/L. Kalicanin *et al.*, 2004 applied potentiometric stripping analysis for determining soluble lead in human teeth. No significant different from those obtained by AAS. Kalicanin and Ajdukovic, 2008 examined teeth samples for determining the content of toxic heavy metals using Potentiometric stripping analysis. It was found that synthetic saliva had no influence on heavy metal ion migration from natural teeth.

Table 1 showcase the current methods utilized for the analysis of heavy metals from human dentine samples. It has been observed that lot of work has been done on atomic absorption spectrophotometer (AAS) followed by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)

Table 1 Current methods used for analyzing heavy metal content in the human teeth

S.No.	Aim	Technique	Sample type	Metal detected	Result	References
1.	To detect the cadmium levels in blood, hair, saliva and teeth samples of Iraqi workers	Atomic Absorption spectrometry	Teeth samples along with hair, saliva and blood	Cd	Increased cadmium concentration was observed among the exposed subject.	Al-Jubouri and Bashbosh, 2012
2.	To study the changes in concentration of some elements in deciduous teeth	Atomic Absorption spectrometry	Deciduous teeth	Mn, Fe, Mg, Cu, K, Cr, Pb, Cd and Ca	Statistically significant decrease in the concentration was observed in teeth of older children as compared to younger ones.	Fischer <i>et al.</i> , 2013
3.	To study the age dependent changes in Pb concentration human teeth	Atomic Absorption spectrometry	Teeth	Pb	The annual increase in the concentration of Pb in tissues of the teeth is approx 0.1 µg/g.	Fischer and Wiechula, 2016
4.	To determine the effect of gender, geographic location and smoking upon metal concentration in human enamel and dentine	Flame Atomic Absorption spectrometry	Human dentine and enamel	Ca, Na, Mg, K, Cu, Zn and Fe	High contents of Ca, Na, Mg, K, Cu, Zn and Fe were found in samples analyzed. Significant differences were observed in Zn content in dentine samples of smokers and non smokers	Olovicic <i>et al.</i> , 2020
5.	To detect lead and cadmium in deciduous teeth samples	Graphite furnace atomic absorption spectrometry	Deciduous teeth, scalp hair, capillary blood	Pb and Cd	A positive correlation was found in between the Pb concentration in teeth and blood.	Barton, 2010
6.	To determine the risk factors associated with the high lead levels in dental enamel	GFAAS	Dental enamel	Pb	Association was found in the dental lead levels and environmental risk factors.	Olympioet <i>et al.</i> , 2010
7.	To determine Cd and Pb in human teeth using new method	dispersive liquid-liquid microextraction preconcentration and GFAAS	Teeth	Cd, Pb	Detection limit for cadmium and lead was found to be 5.6 and 45 ng/L.	Zhang <i>et al.</i> , 2011
8.	To compare the content of trace	ICP-MS	Primary and	Na, Mg, Al, Fe,	It was found that permanent teeth	Amr, 2011

	elements in primary and permanent teeth		permanent teeth	Ni, Cu, Sr, Cd, Ba, Pb and U, Mn, Co, As, Se, Mo and Bi	contained higher concentration of Na, Mg, Al, Fe, Ni, Cu, Sr, Cd, Ba, Pb and U and significantly less Mn, Co, As, Se, Mo and Bi than the children teeth.	
9.	To obtain metal exposure due to environmental pollution in human teeth dentine	ICP-MS	Teeth dentine	As, Mn, Ba, Cu, Cr, Pb, Zn, Hg, Sb, Al, Sr, Sn	It was found that higher metal levels were found in Chinese teeth as compared to Indian and Malay teeth.	Asaduzzaman <i>et al.</i> , 2017
10.	To investigate the concentration of trace elements in human coronal dentine	ICP-MS	Coronal dentine	Pb, Li, Sn, B, Ba, K, Sr, S, Mg, Al, As, Cd, Ca, P, Na, Zn, Fe, Ti, Mn, Cr, Ni, Cu, Co, Se, V	Toxic and essential elements were detected in following order of concentration Al > Pb > Sn > Li > As > Cd and Ca > P > Mg > Na > S > K > Sr > Zn > Ba > Fe > B > Ti > Mn > Cr > Ni > Cu > Co > Se > V	Fernandez-Escudero <i>et al.</i> , 2020
11.	To perform elemental analysis on bone and teeth samples for discrimination purposes	LA-ICP-SF-MS	Teeth samples	Mg, Mn, Fe, Cu, Zn, Rb, Sr, Ba, Pb	It was found that separation of individuals was improved when elemental composition of enamel and dentine, cementum layers were used.	Castro <i>et al.</i> , 2010
12.	To examine relative concentrations of lead, mercury and manganese in enamel regions of deciduous teeth from children with Autism spectrum disorders and high levels of disruptive behavior	LA-ICP-MS	Teeth	Pb, Mn, Hg	No significant differences in levels of neurotoxicants were observed for children with ASDs and HDB.	Abdullah <i>et al.</i> , 2011
13.	To perform the elemental bio-imaging of trace elements in teeth	LA-ICP-MS	Teeth	Pb, Zn, Cd	Concentration of Pb, Zn and Cd was found to be higher in dentine and in the regions adjacent to the pulp.	Hare <i>et al.</i> , 2011

14.	To evaluate the elements migration in human teeth with and without fillings	LA-ICP-MS	Teeth	Al, Ba, La, Sr	Elements like Al, Ba, La, Sr gets migrated from fillings placed in dental cavities to healthy part of teeth.	Hanc <i>et al.</i> , 2013
15.	To study the diet patterns and to detect the trace elements in tooth enamel and dentine samples from Muslim population in Tauste (North Spain)	LA-ICP-MS	Tooth enamel and dentine	Na, Mg, Al, Sc, Ti, V, Cr, Mn, Fe, Co, Cu, Zn, Rb, Sr, Ba and Pb	High Pb content was found in some individuals due to intoxication by occupational exposure to anthropogenic lead.	Guedeet <i>et al.</i> , 2017
16.	To determine the associations between dentine biomarkers of Mn, Zn, Pb and later childhood behaviours	LA-ICP-MS	Teeth	Mn, Pb, Zn	It was reported that prenatal dentine Mn may be protective but excessive postnatal Mn increases the risk for adverse behaviours.	Horton <i>et al.</i> , 2018
17.	To evaluate the differences in lead distribution in different bone and tooth structures	XRF	Teeth	Pb	Highest amount of lead was found in the pulp and root of the tooth structure.	Dias <i>et al.</i> , 2015
18.	To Compare the chemical composition of human teeth with other mammal species	WDXRF	Human and mammal teeth	Mg, S, Sr, Zn, P, Ca, Cl, Cu, K	It was found that human and bovine enamel and dentine species showed the greatest similarity among the other species analyzed.	Terueletal., 2015
19.	To Determine the distribution of toxic elements in teeth treated with amalgam	EDXRF	Teeth	Hg	Higher concentration values of Hg were reported in the teeth treated with amalgam fillings.	Guerra <i>et al.</i> , 2016
20.	To determine whether suspected tooth is authentic human tooth or not	HHXRF	Teeth	Si, S, Ti, Cr, Mn, Fe, Ni, Cu, Zn and Zr	It was found that human teeth can be effectively identified from other species but sex cannot be determined from the teeth samples.	Nganvongpanite <i>et al.</i> , 2017
21.	To develop new method to determine Sr/Ca changes in the enamel of human lower third molar.	Femtosecond Laser Induced Breakdown spectroscopy (fs-LIBS)	Teeth	Sr	Relative amount of strontium was determined.	Alviraet <i>et al.</i> , 2011
22.	To determine the	LIBS	Teeth	Pb, Cd,	The respective	Alhasmiet <i>et al.</i> ,

	levels of toxic elements such as lead, cadmium and arsenic present in roots of extracted teeth of smokers and non smokers			and As	elemental concentration (Pb, Cd, and As) 23. 29, 0.26. 0. 31, and 0.64. 11 ppm was found for nonsmokers, 35. 55, 0.33. 0.51, and 0.91. 1.5 ppm for smokers, and lastly 0.17. 0.31, 0.01. 0.05, and 0.05. 0.09 ppm for control group.	2015
23.	To analyze human deciduous teeth	LIBS	Teeth	Ca, Fe, Sr, Zn and Pb	Elemental concentration of Ca, Fe, Sr, Zn and Pb was found to be highest in enamel, then in dentine and lowest in cementum.	Khalid <i>et al.</i> , 2015
24.	To characterize human teeth	LIBS	Teeth	Ca, F, Si, Zn, Na, Sn, Ar, Li, K, Ce, Fe, Mn, Ti, Al, Cr and P	It was found that Indonesian human teeth contain some elements such as Ca, F, Si, Zn, Na, Sn, Ar, Li, K, Ce, Fe, Mn, Ti, Al, Cr and P.	Suyanto <i>et al.</i> , 2018
25.	To estimate the trace elements in various parts of human teeth	External PIXE	Teeth	P, Ca, V, Mn, Fe, Ni, Cu, Zn, Ba, As, Sr and Pb	Elements including P, Ca, V, Mn, Fe, Ni, Cu, Zn, Ba, As, Sr and Pb were estimated on different parts of human teeth.	Rao <i>et al.</i> , 2010
26.	To analyze elemental profiles of enamel, cementum and caries of human teeth	PIXE	Teeth	P, Ca, V, Mn, Fe, Cu, Zn, As, Sr and Pb	Ten elements were estimated and respective concentration of elements namely P, Ca, Fe, Zn and Pb in enamel is more than those in cementum.	Rautray <i>et al.</i> , 2010

LIMITATION

In forensic casework situations, the exhibits are generally found in trace amount. Therefore, it becomes necessary to employ technique which are non-destructive in

nature. In case of heavy metal detection, majority of the techniques utilized are destructive in nature and also consumes large amount of sample. Also, sampling in case of teeth dentine is problematic

because it has difference in density to accumulate trace elements. Many people undergo various dental treatments which can affect the levels of some trace elements.

CONCLUSION

Analytical techniques including AAS, GFAAS, ICP-MS, LA-ICP-MS, LIBS, XRF, PIXE and electrochemical methods have been employed for the detection of traces of heavy metals present in human teeth. These techniques can effectively help in the determination of different heavy metals which get accumulated in the human teeth due to numerous factors like environmental pollution, anthropogenic trace metal exposure, individual's nutritional status resulting toxic outcomes. Out of all these techniques, XRF could help in the differentiation of the human teeth from other mammal species on the basis of their chemical composition non-destructively. However, atomic absorption spectrometry (AAS) is widely used for the analysis of human teeth whereas electrochemical methods are not extensively used for the detection of heavy metal traces.

In future, work can be done on the detection of heavy metals utilizing non-destructive techniques. Also, there is a need to have reference tooth material which could provide a check on the analytical results from different laboratories. To carry out

biomonitoring studies (environmental or occupational exposure), it is important to determine the concentration of both toxic and essential elements present in the human dentine.

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