Analytical Approach of Different Optical Spectroscopic Techniques to Detect Elemental Profile of Human Teeth Dentine: A Critical Review

Saifullah Jamali¹, Muhammad Aslam Khoso¹, Irfan Ali Sanjrani¹, Hussain Saleem^{3*}, Tariq Ali Siyal¹, Muhammad Ashraf^{1,2}, Mansoor Ahmed Memon¹, Ghulam Murtaza¹, Zahid Hussain Arain¹, Zaheer Ahmed Ujjan¹, Muhammad Niaz Laghari¹, Nek M. Shaikh¹, Waseem A. Bhutto¹, Abdul Majid Soomro¹, Altaf H. Nizamani¹, Samina Saleem⁴

¹Institute of Physics, University of Sindh, Jamshoro, Pakistan.

²Department of Basic Science, Dawood University of Engineering and Technology, Karachi, Pakistan.

³Department of Computer Science, UBIT, University of Karachi, Karachi, Pakistan.

⁴University of Karachi, Karachi, Pakistan.

*Corresponding Author: hussainsaleem@uok.edu.pk

Abstract

Numerous articles on the examination of heavy metals in human teeth have been published in recent years. In today's toxicology area, heavy metal poisoning is a widespread issue. It has been discovered that long-term exposure to heavy metals typically present in traces, in our everyday meals, drinking water, and in the environment as pollution causes heavy metal poisoning in people. Industrial effluents, coal and oil, as well as a variety of consumer items, such as cosmetics, can all cause this type of exposure. Teeth, which are often thought of as exoskeleton parts, store heavy metals with a high affinity and represent long-term exposure information. Instead, then examining the entire tooth, just chosen sections of dentine are examined. We have combined the work done on the examination of heavy metals in human teeth using several instrumental approaches in the current study.

Key words:

Elemental Profile; Heavy metals; Pollution; Spectroscopy; Toxic metals;

1. Introduction

Humans have two sets of teeth at any one moment during their lives. Deciduous/temporary/milk teeth are the name given to one set of teeth. There are a total of 20 teeth in both jaws consisting of four incisors, two canines, and four molars, for a total of ten teeth in each jaw, that erupt at the age of six months and starts shedding at the age of six years [16]. The permanent teeth, which are the second set of teeth, next take their place. There are total 32 teeth in which 16 teeth exist in each jaw, including four incisors, two canines, four premolars, and six molars. Until the age of 22 - 25years, the calcification of the permanent teeth's roots is complete. The crown and root sections of the tooth are the two primary elements [16][34]. The crown of the tooth is visible in the mouth, whereas the root of the tooth is in the jaw and drops beyond the gum line. Enamel, dentine, pulp, cementum, and the periodontal ligament are the most important components of teeth [18].

Enamel, which is made up of rock-hard minerals and calcium phosphate, is the outermost and toughest component of the tooth [12][22][32][51][56][59][67][79][82]. The dentine is a hard tissue that lies underneath the enamel and is filled with tiny channels [25][62]. When cold or heat penetrates the tooth through this channel, enamel is damaged, causing sensitivity or discomfort [24][52]. Pulp is the live, softer middle component of the tooth. Pulp contains nerves, connective tissue, and blood arteries. The connective tissue layer that links the roots to the jawbone and gums is known as cementum [58]. The periodontal ligament is a soft tissue that cushions the tooth against the jaw. Bony and mucosal structures surround the tooth [6][33][57]. Humans have been exposed to increased quantities of heavy metals through both the terrestrial and aquatic environments since the dawn of time [8][53][54]. Metals having large atomic numbers, atomic weights, and densities are known as heavy metal [37][55][68][80]. Some of these metals, such as calcium, magnesium, zinc, and iron, are needed for human health, whereas others, such as cadmium, arsenic, lead, nickel, chromium, copper, and mercury, have been shown to be very hazardous to humans[38][72][73]. Ingestion, inhalation, and dermal contact are all ways for heavy metals to enter the human body [4][26][27].

Heavy metals accumulate in numerous organs, including tissues, bones, and teeth, and their chronic toxicity has an impact on human health [31][44][45]. Toxic metals are slowly taken into the body, but once there, they are difficult to remove and build up in the body permanently [2][60][71]. Heavy metals aid in the management of human body systems such as homeostasis, transport, and binding to cell components due to their chemical coordination and oxidation-reduction capabilities [1][3][43]. By removing native metals from their normal binding sites, they connect with protein sites, producing cell dysfunction and, ultimately, severe toxicity. Heavy metal accumulation varies by organ [41].

The amount of heavy metals absorbed into the body is determined by their binding to carrier molecules. Metallothionein (small proteins) is important for heavy metal storage since it is responsible for the metal binding characteristics [20][50][76]. They can induce hepatotoxicity by forming a cysteine-metallothionein complex in the liver when they bind to cysteine-rich proteins like metallothionein [17][36]. Heavy metals are absorbed into calcified tissues throughout the development stage when they are exposed to heavy metals in their mineral phase. Information regarding recent exposures can be found in urine and blood. Hair and fingernails can represent information on exposure durations ranging from a few months to years, but they can also be polluted by dyes, shampoo, nail paints, airborne dust, and other contaminants [76]. Heavy metals accumulate with strong affinity in calcified tissues (bones and teeth), and this knowledge reflects long-term exposure. Bones are generally available for sample, but teeth provide a lasting record of recent and/or historical heavy metal exposure. Dental tissues are extremely hard, consisting of a substance comparable to that of bones, and are often regarded as an exo-skeleton component [14][77]. Because human teeth are biological tissue that is easily accessible, heavy metal analysis from human teeth has been used to classify individuals in terms of heavy metal absorption and exposure. It is not necessary to investigate the entire tooth; just chosen portions of dentine are required [75].

Human dentine tissues are part of the exoskeleton and do not undergo mineral phase turnover [49]. It has a number of benefits over other bio-indicators, making it a credible indicator of harmful heavy metal exposure in the environment. Because the dentine is surrounded by enamel and cementum, it is unaffected by the oral environment. Odontoblasts are located in the pulp and manufacture dentine constantly until the tooth is lost. Hard tissues of the teeth (dentine and enamel) are more useful than soft tissues such as the kidney and liver because they are not vulnerable to heavy metal changeover once ingested [13][14]. Because metals may pass across the placental barrier, teeth also contain heavy metals ingested by the mother during pregnancy, as their production begins in the prenatal period [31][61]. Shed deciduous teeth can be gathered from dental clinics, schools, or approaching households. Permanent teeth are gathered from dental clinics where they are commonly pulled for orthodontic or other purposes.

Ancient teeth are collected from graveyards [46]. Teeth may be preserved for a long period; the only precaution is to keep them out of an acidic or leaching environment. As a result, calcified tissues on teeth preserve different heavy elements to which they are exposed and give a reliable historical record [19]. Teeth may be used to estimate age, gender, ethnicity, social standing, employment, and habits, therefore their examination is also utilized for identification.

It takes a great deal of effort and care to get accurate results or data through analysis [15][21][48][74]. Laser Induced Breakdown Spectroscopy (LIBS), Atomic Absorption Spectrometry (AAS), Proton Induced X-Ray Emission (PIXE), X-Ray Fluorescence (XRF), Inductively Coupled Plasma (ICP), are some of the instrumental methods used to analyze. The purpose of this study is to look at the current state of elemental analysis from the hard tissue dentine of teeth.

2. Techniques Used for Analysis

Using various chemical analysis techniques and analytical procedures, several investigations have been undertaken to identify heavy metals in human teeth. The discussed analytical procedures were used in the bulk of the investigation.

2.1. Laser Induced Breakdown Spectroscopy (LIBS)

Laser-induced breakdown spectroscopy is a flexible technique for identifying the elements present in the material under investigation. It aids in the identification of metals and non-metals in less than a second with little sample preparation and no waste formation, as well as the detection of exogenous and endogenous substance elements in people and animals.

2.2. Atomic Absorption Spectroscopy (AAS)

It is the most widely used technique for detecting trace elements in various materials, particularly in dental samples.

2.3. Particle Induced X-Ray Emission (PIXE)

Particle Induced X-Ray Emission (PIXE) is a fast and nondestructive approach for simultaneous multi-element analysis with sensitivity in the parts-per-million (ppm) range across the board.

2.4. X-Ray Fluorescence (XRF)

Without inflicting harm to the specimens, XRF gives essential elemental information.

2.5. Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS)

It is one of the most used techniques for elemental analysis since it can deliver high levels of sensitivity, precision, and accuracy. Various scholars have used this approach in a number of investigations.

2.6. Laser Ablation Inductively Coupled Plasma-Mass Spectroscopy (LA-ICP-MS)

This specific approach of laser ablation-inductively coupled plasma-mass spectrometry is beneficial for determining the change of the elemental composition in the sample. This method has been used by several researchers to assess the level of heavy metals in human tooth samples.

3. Literature Review and Discussion

Number of researcher works in this field such as using LIBS, Alhasmi et al., [7] measured the quantities of hazardous metals including lead, cadmium, and arsenic in the roots of removed teeth from smokers and non-smokers. For nonsmokers, the relevant elemental concentrations (Pb, Cd, and As) were 23 - 29, 0.26 - 0.31, and 0.64 - 11 ppm; for smokers, 35 – 55, 0.33 – 0.51, and 0.91 – 1.5 ppm; and for the control group, 0.17 - 0.31, 0.01 - 0.05, and 0.05 - 0.09 ppm. Khalid et al., [47] used LIBS to examine human deciduous tooth samples. Ca, Fe, Sr, Zn, and Pb elemental concentrations were found to be greatest in enamel, followed by dentine, and finally cementum. LIBS was used to classify human teeth by Suyanto et al., [81] Ca, F, Si, Zn, Na, Sn, Ar, Li, K, Ce, Fe, Mn, Ti, Al, Cr, and P have all been discovered in Indonesian human teeth. Alvira et al., [10] established a novel technique for determining Sr/Ca alterations in human lower third molar enamel. The relative quantity of strontium was measured using femtosecond Laser Induced Breakdown spectroscopy (fs-LIBS).

Using AAS, Al-Jubouri et al., [9] found cadmium levels in Iraqi employees' blood, hair, saliva, and tooth samples. The exposed subjects' cadmium levels were found to be higher. Fischer et al., [30] used AAS to investigate variations in the concentration of certain elements (Mn, Fe, Mg, Cu, K, Cr, Pb, Cd, and Ca) in deciduous teeth. Teeth of older children had a statistically significant lower concentration than those of younger children. Fischer et al., [29] used AAS to investigate age-related alterations in human teeth. The yearly rise in Pb content in tooth tissues is around 0.1 g/g. Using Flame Atomic Absorption Spectroscopy (FAAS), Olovcic et al., [64] assess the amount of 12 metals in 23 tooth samples from two cities in Bosnia and Herzegovina. Ca, Na, Mg, K, Cu, Zn, and Fe were detected in abundance in the samples examined. Gender, geographic location, and smoking differences were more pronounced between intra groups than between inter groups. Using GFAAS, Olympio et al., [65] investigated the risk variables linked with elevated lead levels in tooth enamel in 160 adolescents. Using GFAAS, Barton et al., [16] examined 300 samples of 6-year-old children for lead and cadmium in deciduous teeth, scalp hair, and capillary blood. The levels of *Pb* in teeth and blood were shown to have a positive link.

Using external beam PIXE, Rao et al., [69] calculated the trace elements in various regions of human teeth. *P*, *Ca*, *V*, *Mn*, *Fe*, *Ni*, *Cu*, *Zn*, *Ba*, *As*, *Sr*, and *Pb* were measured on various sections of human teeth in this study. Rautray et al., [70] used external PIXE to investigate the elemental profiles of enamel, cementum, and cavities in human teeth. The concentrations of ten elements, namely *P*, *Ca*, *Fe*, *Zn*, and *Pb*, in enamel are higher than those in cementum. Opera et al., [66] studied teeth using PIXE and found *Ca*, *Cr*, *Cu*, *Fe* and *Zn*.

Dias et al., [23] used Micro-X-Ray Fluorescence (MXRF) to investigate changes in lead distribution in various bone and tooth tissues. Pb buildup was also measured ante-mortem and post-mortem. The pulp and root of the tooth structure had the highest level of lead. Guerra et al., [36] used Energy Dispersive X-Ray Fluorescence (EDXRF) to assess the distribution of hazardous components in amalgam-treated teeth. Teruel et al., [78] used Wavelength Dispersive X-Ray Fluorescence (WDXRF) to compare the chemical makeup of human teeth to that of other animal species. Human and bovine enamel and dentine species were determined to be the most species comparable among the other studied. Nganvongpanit et al., [63] suggested a novel approach for evaluating using Hand-Held X-Ray Fluorescence (HHXRF) if a suspected tooth is genuine human teeth or not. It was discovered that human teeth can be distinguished from those of other animals, but that sex cannot be ascertained from tooth samples.

Amr et al., [11] used ICP-MS to compare the content of trace elements in the children's primary and permanent teeth. Permanent teeth had larger concentrations of Na, Mg, Al, Fe, Ni, Cu, Sr, Cd, Ba, Pb, and U than children's teeth, but much less Mn, Co, As, Se, Mo, and Bi. Asaduzzaman et al., [14] used ICP-MS to examine human teeth dentine in order to determine metal exposure owing to pollution. Chinese teeth have greater metal levels than Indian and Malay teeth, according to the study. ICP-MS and Atomic Emission Spectroscopy were used by Escudero et al., [28] to analyze the content of 25 trace elements in 150 human coronal dentine. Both hazardous and important elements were discovered in coronal dentine, with increases in toxic (Pb, Li, Sn) and essential (B, Ba, K, Sr, S, and Mg) concentrations linked to tooth age but independent of sex.

Castro et al., [21] used a sector field based inductively coupled plasma mass spectrometry (LA-ICP-SF-MS) paired with a laser ablation system to perform quantitative analysis on bone and tooth samples. Teeth samples from 14 distinct people were examined, and it was discovered that using the elemental makeup of enamel and dentine, as well as the cementum layers, enhanced individual separation. Using LA-ICP-MS, Abdullah et al., [5] looked the relative quantities of lead, mercury, and manganese in the enamel areas of deciduous teeth from children with Autism Spectrum Disorders and high levels of disruptive behavior. There were no significant variations in neuro-toxicant levels between children with ASDs (Autism Spectrum Disorder) and those with HDB (High Levels of Disruptive Behavior). Hare et al., [40] used LA-ICP-MS to do bio-imaging of trace elements in teeth. Pb, Zn, and Cd concentrations were found to be greater in dentine and the areas close to the pulp. Hanc et al., [39] studies the elements migration in human teeth using LA-ICP-MS and found Al, Ba, La, and Sr. Guede et al., [35] used LA-ICP-MS to analyze 23 teeth enamel and dentine samples from a Muslim community in Tauste (North Spain) in order to discover trace components and examine Medieval Muslim food trends.

The findings revealed that some people had high Pb levels as a result of occupational exposure to anthropogenic lead. The chemical makeup differed by sex and age, and it was shown to be linked to food consumption. Using LA-ICP-MS Horton et al., [42] investigated the links between dentine biomarkers of Mn, Zn, and Pb and later childhood behavior. Prenatal dentine Mn has been shown to be protective, while high postnatal Mn has been shown to increase the chance of negative behaviors. Additionally, increased Mn, Zn, and Pb concentrations have a negative influence on behavior.

4. Conclusion

For the identification of traces of heavy metals present in human teeth, analytical techniques such as AAS, GFAAS, ICP-MS, LA-ICP-MS, LIBS, XRF, PIXE, and electrochemical approaches have been used. These methods can efficiently aid in the identification of various heavy metals that accumulate in human teeth as a consequence of a variety of causes such as environmental pollution, anthropogenic trace metal exposure, and an individual's nutritional state, all of which result in harmful results. XRF, out of all of these methods, has the potential to nondestructively differentiate human teeth from those of other mammalian species based on their chemical composition. However, Atomic Absorption Spectrometry (AAS) is frequently utilized for the examination of human teeth, but electrochemical approaches for the detection of heavy metal residues are not generally employed.

References

- A. H. Nizamani, B. Rasool, M. Tahir, N. M. Shaikh and H. Saleem, "Adiabatic ION Shuttling Protocols in Outer-Segmented-Electrode Surface ION Traps," International Journal of Scientific & Engineering Research (IJSER), vol. 4, no. 6, pp. 3055-3061, 2013.
- [2] A. H. Nizamani, M. A. Rind, N. M. Shaikh, A. H. Moghal and H. Saleem, "Versatile Ultra High Vacuum System for ION Trap Experiments: Design and Implementation," International Journal of Advancements in Research & Technology (IJoART), vol. 2, no. 5, pp. 502-510, 2013.
- [3] A. H. Nizamani, S. A. Buzdar, B. Rasool, N. M. Shaikh and H. Saleem, "Computer-Based Frequency Drift Control of Multiple LASERs in Real-Time," International Journal of Scientific & Engineering Research (IJSER), vol. 4, no. 6, pp. 3038-3045, 2013.
- [4] A. M. Soomro, W. A. Bhutto, A. H. Nizamani, H. Saleem, M. Y. Soomro, M. A. Khaskheli and N. M. Shaikh, "Controllable Growth of Hexagonal BN Monolayer Sheets on Cu Foil by LPCVD," IJCSNS - International Journal of Computer Science and Network Security (ISSN:1738-7906), Web of Science Indexed, HEC Recognized "Y" Category Journal, pp. 85-90, 2019.

- [5] Abdullah, M.M., Ly, A.R., Goldberg, W.A., Clarke-Stewart, K.A., Dudgeon, J.V., Mull, C.G., Chan, T.J., Kent, E.E., Mason, A.Z., and Ericson, J.E., 2011, Heavy metal in children's tooth enamel: Related to autism and disruptive behaviors, J. Autism Dev. Disord., 42, 929 – 936. https://doi.org/10.1007/s10803-011-1318-6
- [6] Agrawal, A., and Tiwari, A.K., 2021, Dentoalveolar Fractures, Max. Tra., 159 – 176.
- [7] Alhasmi, A.M., Nasr, M.M., Gondal, M.A., Shafik, S., and Habibullah, Y.B., 2015, Detection of toxic elements using laser-induced breakdown spectroscopy in smokers; and nonsmokers; teeth and investigation of periodontal parameters, Appl. Opt., 54 (24), 7342 – 7349. https://doi.org/10.1364/AO.54.007342
- [8] Ali, H., Khan, E., and Ilahi, I., 2019, Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation, J. Chem., 6730305. https://doi.org/10.1155/2019/6730305
- [9] Al-Jubouri, R.H., 2012, Assessment of cadmium levels in Blood, hair, saliva and teeth in a sample of Iraqi workers and detection of dental findings, J Bagh Coll. Dent., 24.
- [10] Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M.J., 2010, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, Bioresour. Technol., 101, 4851 – 4861. https://doi.org/10.1016/j.biortech.2009.11.093
- [11] Amr, M.A., 2011, Trace elements in Egyptian teeth, Int. J. Phys. Sci., 6, 6241 – 6245. https://doi.org/10.5897/IJPS09.307
- [12] Andermatt, L., and Ozcan, M., 2021, Micro shear bond strength of resin composite cement to coronal enamel/dentine, cervical enamel, cemento enamel junction and root cementum with different adhesive systems, J. Adhes. Sci. Tech., 35, 2079 2093. https://doi.org/10.1080/01694243.2021.1872195
- [13] Asaduzzaman, K., 2017, Natural radioactivity and heavy metal pollutants in staple foodstuff and human teeth collected from selected areas in peninsular Malaysia, Fac. Sci. Univ. Malaya Kuala Lumpur.
- [14] Asaduzzaman, K., Khandaker, M.U., Binti Baharudin, N.A., Amin, Y.B.M., Farook, M.S., Bradley, D.A., and Mahmoud, O., 2017, Heavy metals in human teeth dentine: A bioindicator of metals exposure and environmental pollution, Chemo., 176, 221 – 230. https://doi.org/10.1016/j.chemosphere.2017.02.114
- [15] Báez, A., Belmont, García, R., Carlos, J., and Báez, A., 2004, Cadmium and Lead levels in deciduous teeth of children living in Mexico city, Rev. Int. Contam. Ambient., 20, xx – xx.
- [16] Barton, H.J., 2011, Advantages of the use of deciduous teeth, hair, and lood analysis for lead and cadmium bio monitoring in children. A study of 6-year-old children from Krakow (Poland), Bio. Trac. Elem. Res., 143, 637 – 658. https://doi.org/10.1007/s12011-010-8896-6

- [17] Batool, J., Amin, N., Jamil, Y., Shaikh, N.M., and Islam, S.A., 2021, Rapid elemental analysis of human teeth using laser induced breakdown spectroscopy, Phys. B. Cond. Mat., 602, 412495. https://doi.org/10.1016/j.physb.2020.412495
- [18] Britannica, "Britannica Encyclopaedia," Encyclopædia Britannica, Inc., 2013. [Online]. Available: https://www.britannica.com/science/tooth-anatomy. [Accessed 2022].
- [19] Budd, P., Montgomery, J., Cox, A., Krause, P., Barreiro, B., and Thomas, R.G., 1998, The distribution of lead within ancient and modern human teeth: Implications for long-term and historical exposure monitoring, Sci. Total Environ., 220, 121 – 136. https://doi.org/ 10.1016/s0048-9697(98)00244-7
- [20] Calisi, A., Lionetto, M.G., De Lorenzis, E., Leomanni, A., and Schettino, T., 2014, Metallothionein induction in the coelomic fluid of the earthworm Lumbricus terrestrosin following heavy metal exposure: A short report, Biomed Res. Int., 109386. https://doi.org/10.1155/2014/109386
- [21] Castro, W., Hoogewerff, J., Latkoczy, C., and Almirall, J.R., 2010, Application of laser ablation (LA-ICP-SF-MS) for the elemental analysis of bone and teeth samples for discrimination purposes, Forensic Sci. Int., 195, 17 – 27. https://doi.org/10.1016/j.forsciint.2009.10.029
- [22] Chatzistavrou, X., Papagerakis, S., Ma, P.X., and Papagerakis, P., 2012, Innovative approaches to regenerate enamel and dentin, Int. J. Dent., 856470. https://doi.org/10.1155/2012/856470
- [23] Dias, A.A., Carvalho, M., Carvalho, M.L., and Pessanha, S., 2015, Quantitative evaluation of ante-mortem lead in human remains of the 18th century by triaxial geometry and bench top micro-X-ray fluorescence spectrometry, J. Anal. At. Spectrom., 30, 2488 2495. https://doi.org/10.1039/C5JA00340G
- [24] Dostalova, T., Jelinkova, H., Remes, M., Šulc, J., and Němec, M., 2016, The use of the Er: YAG laser for bracket debonding and its effect on enamel damage, Photom. Las. Surg., 34, 394 – 9. https://doi.org/10.1089/pho.2016.4115
- [25] Duangto, P., Janhom, A., Prasitwattanaseree, S., Mahakkanukrauh, P., and Iamaroon, A., 2016, New prediction models for dental age estimation in Thai children and adolescents, Forensic Sci. Int., 266, 583.e1 – 583.e5. https://doi.org/10.1016/j.forsciint.2016.05.005
- [26] Duruibe, J.O., and Egwurugwu, M.O.C.O, 2007, Heavy metal pollution and human biotoxic effects, Int. J. Phys. Sci., 2, 112 – 118.
- [27] Engwa, G.A., Ferdinand, P.U., Nwalo, F.N., and Unachukwu, M.N., 2019, Mechanism and health effects of heavy metal toxicity in humans, Pois. Mod. Worl.
- [28] Fernández-Escudero, A.C., Legaz, I., Prieto-Bonete, G., López-Nicolás, M., Maurandi-López, et al., 2020, Aging and trace elements in human coronal tooth dentine, Sci. Rep., 10. https://doi.org/10.1038/s41598-020-66472-1

- [29] Fischer, A., and Wiechuła, D., 2016, Age-Dependent Changes in Pb Concentration in Human Teeth, Biol. Trace Elem. Res., 47 – 54. https://doi.org/10.1007/s12011-016-0643-1
- [30] Fischer, A., Wiechuła, D., and Misztela, C.P., 2013, Changes of concentrations of elements in deciduous teeth with age, Biol. Trace Elem. Res., 154, 427 – 432. https://doi.org/10.1007/s12011-013-9744-2
- [31] Fortes, J., Maria, D., Carceles, P., Luna, A., Laserna, J., 2006, International journal Legal Media, https://doi.org/10.1007/s00414-041-1131-9
- [32] Foster, B.L., 2012, Methods for studying tooth root cementum by light microscopy, Int. J. Oral Sci., 43 (4), 199 – 128. https://doi.org/10.1038/ijos.2012.57
- [33] Galler, K.M., and D'Souza, R.N., 2011, Tissue engineering approaches for regenerative dentistry, Regen. Med., 6, 111 – 124. https://doi.org/10.2217/rme.10.86
- [34] Goran Koch, S.P., 2017, Pediatric dentistry: A clinical approach, John Wiley Sons, 408.
- [35] Guede, I., Zuluaga, M.C., Ortega, L.A., Alonso-Olazabal, A., et al., 2017, Analyses of human dentine and tooth enamel by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to study the diet of medieval Muslim individuals from Tauste (Spain), Microchem. J., 130, 287 – 294. https://doi.org/10.1016/j.microc.2016.10.005
- [36] Guerra, M., Ferreira, C., Carvalho, M.L., Santos, J.P., and Pessanha, S., 2016, Distribution of toxic elements in teeth treated with amalgam using μ-energy dispersive X-ray fluorescence, Spectrochim. Acta Part B At. Spectrosc., 122, 114 – 117. https://doi.org/10.1016/j.sab.2016.06.006
- [37] H. Saleem, A. H. Nizamani, W. A. Bhutto, A. M. Soomro, M. Y. Soomro and A. Toufik, "Two Dimensional Natural Convection Heat Losses from Square Solar Cavity Receiver," IJCSNS - International Journal of Computer Science and Network Security (ISSN:1738-7906), Web of Science Indexed, HEC Recognized "Y" Category Journal, pp. 293-298, 2019.
- [38] H. Saleem, M. Z. A. Khan and S. Afzal, "Review of Various Aspects of Radio Frequency IDentification (RFID) Technology," International Organization for Scientific Research - IOSR Journal of Computer Engineering (IOSR-JCE), vol. 8, no. 1, pp. 1-6, 2012.
- [39] Hanć, A., Olszewska, A., and Barałkiewicz, D., 2013, Quantitative analysis of elements migration in human teeth with and without filling using LA-ICP-MS, Microchem. J., 110, 61 – 69. DOI: 10.1016/j.microc.2013.02.006
- [40] Hare, D., Austin, C., Doble, P., and Arora, M., 2011, Elemental bio-imaging of trace elements in teeth using laser ablation-inductively coupled plasma-mass spectrometry, J. Dent., 39, 397 – 403. https://doi.org/10.1016/j.jdent.2011.03.004
- [41] Honda, K., Sahrul, M., Hidaka, H., and Tatsukawa, R., 1983, Organ and tissue distribution of heavy metals, and

their growth-related changes in antarctic fish, pagothenia borchgrevinki, Agric. Biol. Chem., 47, 2521 – 2532. https://doi.org/10.1080/00021369.1983.10865986

- [42] Horton, M.K., Hsu, L., Henn, B.C., Margolis, A., Austin, C., et al., 2018, Dentine biomarkers of prenatal and early childhood exposure to manganese, zinc and lead and childhood behavior, Environ. Int., 121, 148 – 158. https://doi.org/10.1016/j.envint.2018.08.045
- [43] Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., and Beeregowda, K.N., 2014, Toxicity, mechanism and health effects of some heavy metals, Inter. Toxicol., 7, 60. https://doi.org/10.2478/intox-2014-0009
- [44] Jamali, S., Bhutto, W.A., Nizamani, A.H., Saleem, H., Khaskheli, M.A., Soomro, A.M., Sahito, A.G., Shaikh, N.M., Saleem, S., 2019, Spectroscopic Analysis of Lithium Fluoride (LiF) using Laser Ablation, IJCSNS, 19 (8), 127-134. http://paper.ijcsns.org/07_book/201908/20190819.pdf
- [45] Jan, A.T., Azam, M., Siddiqui, K., Ali, A., Choi, I., and Haq, Q.M.R., 2015, Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants, Int. J. Mol. Sci., 16, 29592 – 29630. https://doi.org/10.3390/ijms161226183
- [46] Kamalak, H., Canbay, C.A., Yigit, O., and Altin, S., 2018, Physico-mechanical and thermal characteristics of commercially available and newly developed dental flowable composites, J. Mol. Struct., 1156, 314 – 319. https://doi.org/10.1016/j.jdent.2014.05.009
- [47] Khalid, A., Bashir, S., Akram, M., and Hayat, A., 2015, Laser-induced breakdown spectroscopy analysis of human deciduous teeth samples, Lasers Med. Sci., 309 (30), 2233 – 2238.
- [48] Kolmas, J., Kalinowski, E., and Wojtowicz, A., 2010, Midinfrared reflectance micro spectroscopy of human molars: chemical comparison of the dentine-enamel junction with the adjacent tissues, J. Mol. Struct., 1-3 (966), 113 – 121. https://doi.org/ 10.1016/j.molstruc.2009.12.023
- [49] Koubi, G., Colon, P., Franquin, J.C., Hartmann, A., Richard, G., Faure, M.O., and Lambert, G., 2013, Clinical evaluation of the performance and safety of a new dentine substitute, biodentine, in the restoration of posterior teeth - a prospective study, Clin. Oral Investig., 17, 243 – 249. https://doi.org/10.1007/s00784-012-0701-9
- [50] Li, L.S., Meng, Y.P., Cao, Q.F., Yang, Y.Z., Wang, F., Jia, H.S., Wu, S.B., and Liu, X.G., 2016, Type 1 metallothionein (ZjMT) is responsible for heavy metal tolerance in Ziziphus jujuba, Biochem., 81, 565 – 573. https://doi.org/10.3390/ijms160816750
- [51] Li, Z., Liu, L., Wang, L., and Song, D., 2021, The effects and potential applications of concentrated growth factor in dentin – pulp complex regeneration, Stem, Cell Ther., 12, 1 – 10. https://doi.org/10.1186/s13287-021-02446-y
- [52] Lin, M., Xu, F., Lu, T.J., and Bai, B.F., 2010, A review of heat transfer in human tooth – experimental characterization and mathematical modeling, Dent. Mater., 26, 501 – 513. https://doi.org/10.1016/j.dental.2010.02.009

- [53] M. A. Khoso, A. H. Nizamani, H. Saleem, A. M. Soomro, W. A. Bhutto, S. Jamali, M. S. Kalhoro and N. M. Shaikh, "Elemental Composition of the Soils using Laser Induced Breakdown Spectroscopy," International Journal of Advanced Research in Engineering & Technology (IJARET), ISSN: 0976-6499, Scopus Indexed, HEC Recognized "Y" Category Journal, vol. 12, no. 4, pp. 389-400, 2021.
- [54] M. Y. Channa, A. H. Nizamani, A. M. Soomro, H. Saleem, W. A. Bhutto, M. Y. Soomro, M. A. Khaskheli and N. M. Shaikh, "Vertical Ion Shuttling Protocols for Multi-Strip Surface Ion Traps," IJCSNS - International Journal of Computer Science and Network Security (ISSN:1738-7906), Web of Science Indexed, HEC Recognized "Y" Category Journal, pp. 16-23, 2019.
- [55] M. Y. Channa, A. H. Nizamani, H. Saleem, W. A. Bhutto, A. M. Soomro and M. Y. Soomro, "Surface Ion Trap Designs for Vertical Ion Shuttling," IJCSNS - International Journal of Computer Science and Network Security (ISSN:1738-7906), Web of Science Indexed, HEC
- [56] Maeda, H., 2020, Aging and senescence of dental pulp and hard tissues of the tooth, Front Cell Dev. Bio., 8, 1417. https://doi.org/10.3389/fcell.2020.605996
- [57] Mohammadi, A., Hassani, A., and Fazlisalehi, O., 2021, Use of buccal fat pad in facial cosmetic surgery, Integr. Proced. Facial Cosmet. Surg., 887 – 901.
- [58] Morgenthal, A., Zaslansky, P., and Fleck, C., 2021, Cementum thickening leads to lower whole tooth mobility and reduced root stresses: An in silico study on aging effects during mastication, J. Struct. Biol., 213, 107726. https://doi.org/10.1016/j.jsb.2021.107726
- [59] Nakano, Y., Denbesten, P., and Goldberg, M., 2021, Structure of collagen derived mineralized tissues (dentin, cementum and bone) and non-collagenous extra cellular matrix of enamel, Extr. Mat. Bio. Dent. Tiss. Struct., 10, 3 – 34.
- [60] Nawrocka, A., Piwonski, I., Sauro, S., Porcelli, A., Hardan, L., and Szymanska, M.L., 2021, Traditional microscopic techniques employed in dental adhesion research -Applications and protocols of specimen preparation, Biosens., 11, 408. https://doi.org/10.3390/bios11110408
- [61] Neeti, K., Prakash, T., 2013, Effects of Heavy Metal Poisoning during Pregnancy, Int. Res. J. Environ. Sci., 2, 88 – 92.
- [62] Nganvongpanit, K., Buddhachat, K., Piboon, P., Euppayo, T., and Mahakkanukrauh, P., 2017, Variation in elemental composition of human teeth and its application for feasible species identification., Foren. Sci. Int., 271, 33 – 42. https://doi.org/10.1038/srep46167
- [63] Nganvongpanit, K., Soponteerakul, R., Kaewkumpai, P., Punyapornwithaya, V., Buddhachat, K., et al, 2017, Osteoarthritis in two marine mammals and 22 land mammals: learning from skeletal remains, J. Anat., 231, 140 – 155. https://doi.org/10.1111/joa.12620

- [64] Olovčić, A., Ramić, E., and Memić, M., 2019, Human enamel and dentin: Effect of gender, geographic location and smoking upon metal concentrations, Clin. Analys., 53, 245 – 261. https://doi.org/10.1080/00032719.2019.1646753
- [65] Olympio, K.P.K., Naozuka, J., Oliveira, P.V., Cardoso, M.R.A., Bechara, E.J.H., and Günther, W.M.R., 2010, Association of dental enamel lead levels with risk factors for environmental exposure, Rev. Saudi Publica., 44, 851–858.
- [66] Oprea, C., Szalanski, P.J., Gustova, M.V., Oprea, et al, 2009, XRF detection limits for dental tissues of human teeth, Vacuum., 83, S166 – S168. https://doi.org/10.1016/j.vacuum.2009.01.054
- [67] Ovy, E.G., Romanyk, D.L., Nir, C.F., and Westover, L., 2021., Modelling and evaluating periodontal ligament mechanical behaviour and properties: A scoping review of current approaches and limitations, Orth. Craniofac. Res. https://doi.org/10.1111/ocr.12527
- [68] R. Chand, Saeeduddin, M. A. Khaskheli, A. M. Soomro, H. Saleem, W. A. Bhutto, A. H. Nizamani, M. Y. Soomro, N. M. Shaikh and S. V. Muniandy, "Fractal Analysis of Light Scattering Data from Gravity-Driven Granular Flows," IJCSNS - International Journal of Computer Science and Network Security (ISSN:1738-7906), Web of Science Indexed, HEC Recognized "Y" Category Journal, pp. 83-89, 2019.
- [69] Rao, A.P.R.V., 2010, Estimation of trace elements in various parts of human teeth using external beam PIXE, IJPA., 2, 123 – 134.
- [70] Rautray, T.R., Das, S., and Rautray, A.C., 2010, In situ analysis of human teeth by external PIXE, Nucl. Inst. Meth. Phys. Res. Sect. Beam Interact. Mater. Atoms., 268, 2371 – 2374. https://doi.org/10.1016/j.nimb.2010.01.004
- [71] S. A. Buzdar, M. A. Khan, A. Nazir, M. Gadhi, A. H. Nizamani and H. Saleem, "Effect of Change in Orientation of Enhanced Dynamic Wedges on Radiotherapy Treatment Dose," International Journal of Advancements in Research & Technology (IJoART), vol. 2, no. 5, pp. 496-501, 2013.
- [72] S. Afzal, M. Z. A. Khan and H. Saleem, "A Proposed OEIC Circuit with Two Metal Layer Silicon Waveguide and Low Power Photonic Receiver Circuit," International Journal of Computer Science Issues (IJCSI), vol. 9, no. 5(1), pp. 355-358, 2012.
- [73] S. Afzal, M. Z. A. Khan and H. Saleem, "A Proposed Silicon Optical Electronic Integrated Circuit with Monolithic Integration of LED, OPFET and Receiver Circuit," International Organization for Scientific Research - Journal of Computer Engineering (IOSR-JCE), vol. 6, no. 4, pp. 42-46, 2012.
- [74] S. M. A. Burney and H. Saleem, "Inductive and Deductive Research Approach," University of Karachi, Karachi, 2008.
- [75] Sahebi, S., Moazami, F., and Abbott, P., 2010, The effects of short-term calcium hydroxide application on the strength of dentine, Dent. Traumatol., 26, 43 – 46. https://doi.org/10.1111/j.1600-9657.2009.00834.x

- [76] Samuel, M.S., Datta, S., Khandge, R.S., and Selvarajan, E., 2021, A state of the art review on characterization of heavy metal binding metallothionein's proteins and their widespread applications, Sci. T. Environ., 775. https://doi.org/10.1016/j.scitotenv.2021.145829
- [77] Sharma, G.K.S., 2020, Trend in the analysis of heavy metals in human teeth dentine: a review, J. Indo-Pacific Acad. Forensic Odontol., 93 – 112.
- [78] Teruel, J.D.D., Alcolea, A., Hernández, A., and Ruiz, A.J.O., 2015, Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth, Arch. Oral Biol., 60, 768 – 775. https://doi.org/10.1016/j.archoralbio.2015.01.014
- [79] Trenouth, M.J., 2015, The origin of the terms enamel, dentine and cementum, R. Coll. Surg. Engl., 5, 26 – 31. https://doi.org/10.1308/204268514X13859766312638
- [80] W. A. Bhutto, A. M. Soomro, A. H. Nizamani, H. Saleem, M. A. Khaskheli, A. G. Sahito, R. Das, U. A. Khan and S. Saleem, "Controlled Growth of Zinc Oxide Nanowire Arrays by Chemical Vapor Deposition (CVD) Method," IJCSNS - International Journal of Computer Science and Network Security (ISSN:1738-7906), Web of Science Indexed, HEC Recognized "Y" Category Journal, pp. 135-141, 2019.
- [81] Zaytsev, S.M., Popov, A.M., Zorov, N.B., Suyanto, H., Trisnawati, N.L.P, Putra, I.K., and Suprihatin, I.E., 2018, Characterization of human teeth by laser-induced breakdown spectroscopy, J. Phys. Conf. Ser., 1120, 012018.
- [82] Zhai, Q., Dong, Z., Wang, W., Li, B., and Jin, Y., 2018, Dental stem cell and dental tissue regeneration, Front. Med., 132 (13), 152 – 159. https://doi.org/10.1007/s11684-018-0628-x.