Study of some Trace Elements in Sound and Carious Enamel of Primary and Permanent Teeth of Children in Benghazi City

By

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Faculty of Dentistry

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University of Benghazi **Faculty of Dentistry**

Department of Pediatric Dentistry

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بسم الله الرحمن الرحيم

{وَقُلْ رَّبِّ زِدْنِي عِلْمًا}

[سورة طه : الآية 114]

صدق الله العظيم

DECLARATION

I confirm that this thesis is a record of research carried out by myself, undertaken at the University of Benghazi. Except where otherwise stated the research design and analysis was my own work, subject to the help and advice received from those acknowledged. I have consulted all the reference cited. This research has not previously been submitted for a high degree.

Name: Omima Abdulhamid Omran

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DEDICATION

This work

is dedicated

to my beloved

Father soul

ACKNOWLEDGMENT

First and foremost, praises and thanks to the God, for giving me the power throughout my research work to complete the research successfully.

A few lines are too short to make a complete account of my deep appreciation for all the individuals for their assistance in completing my studies and research. I would like to thank my supervisor Professor ***Nagat Hassan Bubteina*** for offering me a great opportunity to work on this interesting project with her. I want to extend my appreciation for her patience, great understanding, and for the lessons she gave me on the importance of details for the success of my work on this project, and without her guidance and persistent help this dissertation would not have been possible.

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LIST OF ABBREVIATIONS

Abbreviation	Full term
HA/HAP	Hydroxyapatite
Ag	Silver
Al	Aluminum
Au	Gold
B	Boron
Ba	Barium
Be	Beryllium
Bi	Bismuth
Br	Bromine
C	Carbon
Ca	Calsium
Cd	Cadmium
Ce	Cerium
Cl	Chlorine
Co	Cobalt
Cr	Chromium
Cs	Caesium
Cu	Copper
F	Fluoride
Fe	Iron
I	Iodine
Ir	Iridium
K	Potassium

Li	Lithium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Nb	Niobium
Nd	Neodymium
Ni	Nickel
O	Oxygen
P	Potasium
Pb	Lead
Pr	Praseodymium
Pt	Platinum
Rb	Roentgenium
Sb	Antimony
Se	Selenium
Si	Silicon
Sn	Tin
Sr	Strontium
Ti	Titanium
V	Vanadium
Y	Yttrium
Yb	Ytterbium
Zn	Zinc
Zr	Zirconium
TEs	Trace Elements

ISE	Ion -selective electrode
HCL	Hydrochloric acid
TISAB	Total ionic strength adjustment buffer
AAS	Atomic Absorption Spectroscopy
AES	Atomic Emission Spectroscopy
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LA-ICP-MS	laser ablation Inductively Coupled Plasma Mass Spectroscopy
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
FAAS	Flame Atomic Absorption Spectroscopy
ETA –AAS	Electro-Thermal Atomization-Atomic Absorption Spectroscopy
HAP	Calcium hydroxyapatite crystals
Mm	Millimeter
Mm	Micrometer= one million of meter
Ppm	Part per million
Mg	Milligram
WHO	Wold Health Organization
g	Gram
Wt	Wight
Mg	Microgram
PIXE	Particle-Induced X-ray Emission
EDS Rx	Energy Dispersive X-Ray Spectrometer
FHAP	Fluorhydroxyapatite
FAP	Fluorapatite
OH	Hydroxyde

pH	Potential of hydrogen
mmol/L	Milimole/Liter
ART	Arthritis
OST	Osteoporosis
CMRDI	Central Metallurgical Research & Development Institute
TISAB	Total Ionic Strength Adjustment Buffer

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Study of some Trace Elements in Sound and Carious Enamel of Primary and Permanent Teeth of Children in Benghazi City

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Back ground: Dental enamel is the hardest structures in human body that covered the anatomical crown of the tooth and it contains a large number of minor inorganic constituents and trace elements in a varying quantity. The importance of the trace elements in the prevention and reduction of dental caries has not yet been established fully although various studies had been performed to find the link between trace elements and dental caries in human teeth.

Aim: Estimation of some trace elements in sound and carious enamel of primary and permanent teeth of children in Benghazi and evaluation of correlation between the presence of caries and these trace elements.

Materials and Methods: The concentrations of the 18 trace elements, F, Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se, Pb, Mo, and V were estimated by ICP-MS, in a sample composed of 48 sound and caries permanent and primary teeth which were collected from patients attended to different private and governmental dental clinics in Benghazi city. The collected powder samples were divided into four groups (A, B, C, and D) with 12 samples included in each group according to the type of the teeth whether permanent or primary and the conditions of the teeth either sound or caries.

Results: The study revealed that the most common trace elements detected in both sound and carious permanent teeth in this study were Sr, followed by K, F and Al while, the least observed trace elements were Mg, Cr, Co & Cu. The results also showed that the most common trace elements found in both sound and carious primary teeth were Sr, followed by K,F and B while the least observed trace elements were Mg, Cr, Co & Fe. By comparing the results, it was concluded that sound primary teeth had significantly higher amount of Mn, V, B, Si, Fe, Cu & Ni ($p<0.05$). Sound permanent teeth have higher amount of Al, Sr, Cr, F, K & Mg ($p<0.05$), while caries primary teeth showed higher amount of V & Cu ($p<0.05$). Caries permanent teeth had significantly higher amount of Al, Sr, B, Mn, K, F Ni, Fe & Cr ($p<0.05$).

Conclusion: The results of this study support the previous studies about the importance of fluoride as a protective factor against dental caries. Furthermore, beside F, both V and Mn elements may have a cariostatic effect. Al could be cariostatic since it was present in high concentration in sound primary teeth but caries promoting element in permanent teeth. Also B could have a cariostatic effect on primary teeth, whereas Si could formulated as carious predisposing element in permanent teeth and cariostatic element in primary teeth.

Keywords: Enamel, Trace elements, Dental caries.

1. .Introduction

In the earlier part of twentieth century, scientists discovered that human body comprises of several mineral elements which were existed in trace amounts with unknown concentrations and that is why they were named as trace elements.[1]

Recent advances in analytical technologies, such as the development of atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectroscopy (ICP-MS) and others, have made it possible to measure these elements precisely and determined their concentration, functions and the characteristics of their deficiency and excess state.[2] Modes of human trace element intake could be diverse ranging from the normal ingestion of food and drinking of water, to the inhalation from the atmosphere or through dermal absorption.[3] The presence and/or absence of trace elements in the environment will influence the availability of such elements to man.[4]

Although, these elements account for only 0.02% of the total body weight, they are very essential for proper functioning of the vital processes of the body and play an important role on the oral health particularly human teeth.[5] Various diseases of previously unknown etiology have been attributed to an imbalance of trace elements. Both deficiency and excess of trace elements, resulting from exposure to the entire natural and manmade environment, have been associated with many diseases and disorders including dental caries and many other oral diseases, leading to a wide variety of clinical effects.[4] On the other hand, it was reported that the ratio and the distribution pattern of these minerals in the teeth depend on several factors such as the geographic region, the underground water and superficial water, the type of food, the physical abrasion, the application of mechanical strength, the age, the oral care, and the general health of the patient in addition to the environmental factors.[3] Because of the fundamental importance of trace elements in a number of biochemical and physiological processes in humans, it is reasonable to postulate that they may influence mechanisms that are responsible for caries progress or inhibition which is considered the most common oral disease and the major cause of tooth loss and as the third among non-communicable disease that endangers human health.[4]

Since dental enamel is highly mineralized structure that covered the anatomical crown of the tooth which made it directly exposed to the oral environment, it was postulated that the chance of accumulation of trace elements in this tissue is higher than that of the underlying dentine-pulp complex.[6] Enamel consists of 95 % inorganic material in the form of calcium hydroxyapatite crystals (HAP), 2 % organic material such as amylogenic, enameline, ameloblastin, and tuftelin, among others, and 3 % water.[7] The HAP crystals contain various inorganic trace elements which are present in less than 0.01% (i.e., in micrograms per gram). These trace elements are derived from the environment during mineralization and during and after tooth maturation.[8] The presence of sequential calcifying growth layers with fixed composition in enamel structure can provide an archival record of these environmental changes.[9] It was demonstrated that the presences of these elements cause changes in the physical and chemical properties of enamel, where it has been found that the receptivity of the apatite lattice to a wide variety of elements such as strontium, magnesium, lead, zinc, and fluoride facilitates their incorporation into the crystals, if they are present during enamel formation. Such reactions occur principally in the superficial layers of the enamel where the substitution of new elements can influence the solubility of the enamel by enhancing remineralization of enamel, thereby modifying the resistance to caries. [10,11] All these changes that reproduced by the presence of these trace elements make the enamel composition very variable and also, been associated with differences in the prevalence of human dental caries.[10] So, it was stated that the solubility of the enamel to acid attacks depends on the highest or lowest amount of these trace elements in dental caries which act probably through altering the resistance of the teeth itself or by modifying the local environment or the chemical and physical composition of the teeth.[11,12]

A total of 19 trace elements were detected in the tooth enamel samples by ICP with different concentration[11], while others revealed the presence of 35 trace elements in sound enamel of permanent teeth with wide variations in their concentration among different ethnic groups.[13]

Trace elements in tooth enamel have been investigated for their role in caries process as several epidemiological studies have proved that a trace element such as fluoride can alter the solubility of dental tissue and transforming the size and shape of the crystals which makes enamel more resistant to the caries.[3,5,8]

Others found out that the presences of F, Al, Fe, Se and Sr were associated with the low risk of tooth caries, while Mn, Cu and Cd had been associated with a high risk.[14] On the other hand, a different study showed that the correlation between the amounts of Al, Br, Cu, and Z in enamel caries was not significant[15], while a negative correlation was observed between strontium and enamel caries in extra study.[16] An additional study concluded that some other trace elements besides fluoride play an important role in the caries process.[17] Other interesting study showed a reduction in caries prevalence in both deciduous and permanent dentitions with increasing concentrations of V in drinking water.[18]

So, according to the previously mentioned contents, teeth are reported to be suitable indicators for exposure and accumulation of a variety and a wide range of trace elements. It is very interesting to provide a historic record of the elemental characterization in dental enamel to evaluate the trace element concentrations and their relation to caries occurrence in both primary and permanent teeth.

Hence, this retrospective study was carried out to estimate the concentrations of 18 trace elements in carious and intact enamel structure in primary and permanent teeth by using inductive coupled plasma-optic emission spectrometry (ICP-OES) and Fluoride Selective Electrode (ISE).

2. **Review of Literature**

2.1 Delineate of tooth anatomy

A tooth consists of calcified structures namely enamel, dentin, and cementum and a soft tissue which identified as dental pulp tissue exists in a pulp cavity in the center of the tooth through which the dental pulp called the nerve, runs.(19) The portion of a tooth exposed to the oral cavity is known as the dental crown, and the portion below the dental crown is known as the tooth root. In order to receive an impact on the tooth and to absorb and alleviate the force on the jaw, the surface of the tooth root area (cementum) and the alveolar bone are connected by a fibrous tissue called the periodontal ligament. The tooth is supported by the tissue consisting of the alveolar bone, gums and the periodontal ligament.(20)

2.2 An overview of dental enamel

Enamel is a porcelain-like cap covering the clinical crown of the tooth which is the exposed part to the oral environment.(21) Moreover, enamel is formed in a non-uniform layer and its thickness varies from about 0.1 mm in the cervical area to about 2 mm in the occlusal region.(22) From the functional and esthetic point of view, the enamel is considered as one of the most important structures of the tooth as it provides the shape and contour to the crown of tooth and it is the hardest and most resistant tissue in the human body.(23) It protects the dentine-pulp complex from mechanical and chemical impact but unfortunately, it does not has the capacity to remodel or regrow after damage.(19)

2.2.1. Differences between primary and permanent dental enamel

Apparently, although primary and permanent teeth have comparable structure and chemical compositions, there are several important differences between them as clear in (Table 2.1). Some of these differences are observed by visual inspection such as, size or number which are 20 versus 32 for primary and permanent respectively. Also microstructurally, the crystallites in primary teeth have a less organized pattern and tend to be somewhat larger than permanent teeth.[24,25] Evermore, it contains considerable amounts of 'prismless' enamel at the surface of primary enamel which increases its permeability.[26] Chemically, it was proved that both primary and permanent enamel is composed predominantly of calcium deficient carbonato-hydroxyapatite.[27] Various studies indicated that the presence of carbonate in the primary enamel is greater in amounts than in permanent enamel and hence, it increases its solubility.[28,29] Deciduous enamel appears milky white comparing to the permanent enamel because, it has a more opaque crystalline form and the degree of mineralization is much lower than that of permanent teeth with a thickness varies from 0.5-1mm.[21]

Table (2.1): The different between primary and permanent teeth.[20]

	Primary dentition	Permanent dentition
Number	A total of 20 teeth. 10 in each jaw, 5 teeth in each quadrant. There are 2 incisors, 1 canine, 2 molars in each quadrant.	A total of 32 teeth. 16 in each jaw, 8 teeth in each quadrant. There are 2 incisors, 1 canine, 2 premolars and 3 molars in each quadrant.
Size	Primary teeth are smaller in overall size.	Larger in overall dimension.
Color	Lighter in color. They appear bluish-white (milky white)	Permanent teeth are darker in color. They appear yellowish, white or greyish white.

Shape	Crowns of primary teeth are wider mesiodistally in comparison to their crown height.	Crowns of permanent anterior teeth appear longer as their cervicoincisal height is greater than mesiodistal width.
	• Deciduous molars are more bulbous & with marked cervical constriction.	Permanent molars have less constriction of neck
	They have narrow occlusal tables in buccolingual dimensions.	There is less convergence of buccal & lingual surfaces of molars towards occlusal surface. Thus, have broader occlusal table.
Root	Roots of primary teeth are proportionately longer & more slender in comparison to crown size with narrower mesiodistally width.	Permanent roots are shorter & bulkier in comparison to their crown and broader mesiodistally width.
	Furcation of molar roots is placed more cervically so that the root trunk is much smaller. Roots of primary molars flare out markedly from cervical area to their tips(to accommodate permanent tooth buds between their roots).	Furcation in permanent molars is placed more apically & thus root trunk is larger. Absent of root flare out.
	Primary roots undergo physiologic resorption & the primary teeth are shed naturally.	No Physiologic resorption.
Pulp	Pulp chambers of deciduous teeth are proportionately larger when compared to crown size.	Pulp chamber is smaller in relation to crown size.
	Pulp horns of deciduous molars especially mesial horns are higher & closer to outer surface than that of permanent molars.	Pulp horns are comparatively lower & away from outer surface.
	Accessory canals in pulp chambers of primary molars directly lead to inter-radicular furcation areas.	Floor of the pulp chamber does not have many accessory canal.

Enamel	Enamel is thinner. with about 1mm thick but of uniform thickness. The enamel rods at the cervical third are directed horizontally instead of gingivally.	Enamel is 2-3 mm thick & is not uniform in thickness. Enamel rods at the cervix are directed apically.
Dentin	Dentin thickness is half that of permanent teeth. Dentinal tubules are less regular.	Greater thicknesses of dentin over pulpal roof dentinal tubules are more regular.
Cementum	Cementum is thin & made up of only primary cementum. This shows that permanent teeth are firmly anchored in alveolar bone more than primary teeth.	Cementum is thick. Both primary & secondary cementums are present.
Mineral content	Mineral contents- both enamel and dentin are less mineralized and less dense. Neonatal lines are present in all primary teeth both in enamel and dentin.	Enamel and dentin are more mineralized. Neonatal lines are seen only in 1st molar since mineralization begins at birth.

2.2.2. Composition and structure of human enamel

Histologically, dental enamel has a homogenous structure composed of prisms or rods, which are formed during the amelogenesis process, where the ameloblasts begain the formation of the organic matrix first which is considered as a template for the deposition of the inorganic minerals subsequently. These prisms measure approximately 4 µm in diameter and run in parallel bundles from the dentino-enamel junction to the anatomical surface, each being composed of clusters of small crystallites, and the spaces between the prisms and the crystallites are filled with water and organic material such as protein and lipids.(7)

Chemically, mature enamel is an acellular calcified tissue with a high mineral level of approximately 96 % by weight in the form of calcium hydroxyapatite crystal (HAP) which has a chemical formula $Ca_{10}(PO_4)_6(OH)_2$, moreover, it contains 1 % organic substances such as amylogenic, enameline, ameloblastin, and tuftelin, among others, and 3 % of water.(30,31) The HAP crystals contain various inorganic elements in trace amounts usually less than 0.01 % in micrograms per gram and are called the trace elements, where the teeth are accepted as an appropriate indication of these TEs.(32) Scientifically, it was proved that the presence of small amount of trace elements TEs causes variations in the arrangement and the size of the apatite crystals of dental enamel which correspondingly change its physical properties and produces a significant effects on both oral dental health and human health in general.(5,23)

2.3. What are the trace elements?

It is well known that 96 % of the life materials consist of carbon, hydrogen, and nitrogen elements and 50 % of the known elements are at measurable concentrations in the life system.(3) In humans and other mammals, physiological activities of 23 elements are known in which out of them 11elements are classified as trace elements (TEs) (33). TEs consist of transition elements which are vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), and molybdenum(M) and non-metal elements including selenium (Se), fluorine (f), and iodine(I). These trace elements fall into the micro-nutrient category, which is required at negligible levels of less than 100 mg/ day, unlike sodium(Na), calcium(Ca), magnesium(Mg), potassium(K), and chlorine(Cl) which are considered as macronutrients since they are required at larger amounts.(3) Both of major and trace elements play an essential role in human health and the lack or abundance of these elements due to natural or man-made reasons can lead to critical clinic consequences. (34)

2.3.1. Definition:

Generally, there are different definitions attributed to the trace element depend on the field of science:

In analytical chemistry: a trace element is the one in which the average concentration is less than 100 parts per million (ppm) measured in the atomic count.[35]

In biochemistry, the trace elements are defined as a dietary elements that are needed in very minute quantities for the proper growth, development, and physiology of the organism.[36]

2.3.2. Classification of the trace elements

Different classifications have been introduced based on different strategies such as:

- **Biological classification of trace elements based on their amount in tissues:**

Table(2.2): (A-C) Different classifications for Trace Elements.[36]

A. World Health Organization classification of elements (1973).[37]	
Essential elements	Chromium, Copper, Selenium, Molybdenum, Iodine and Zinc.
Probably essential elements	Manganese, Silicon, Nickel, Boron and Vanadium.
Potentially toxic elements	Fluoride, Lead, Cadmium, Mercury and Lithium.

B. Frieden's Categorical Classification of Elements (1974). [38]	
Group I	Basic components of macromolecules such as carbohydrates, proteins, and lipids. Examples include carbon, hydrogen, oxygen, and nitrogen.
Group II	Nutritionally important minerals also referred to as principal or macroelements. The daily requirement of these macroelements for an adult person is above 100 mg/day. Examples include sodium, potassium, chloride, calcium, phosphorous, magnesium, and sulfur.
Group III	Essential trace elements. These trace elements are also called minor elements. An element is considered a trace element when its requirement per day is below 100 mg. The deficiency of these elements is rare but may prove fatal. Examples include copper, iron, zinc, chromium, cobalt, iodine, molybdenum, and selenium.
Group IV	Additional trace elements. Their role is yet unclear and they may be essential. Examples include cadmium, nickel, silica, tin, vanadium, and aluminum. This group may be equivalent to probably essential trace elements in the WHO classification.
Group V	These metals are not essential and their functions are not known. They may produce toxicity in excess amounts. Examples include gold, mercury, and lead. This group is equivalent to potentially toxic elements defined in the WHO classification.

C- Frieden's Classification of Elements (1981) based on their amount in human tissues. [39]	
Essential elements	Boron, Cobalt, Copper, Iodine, Iron, Manganese, Molybdenum, Zinc.
Probably essential elements	Chromium, Fluorine, Nickel, Selenium, Vanadium.
Potentially toxic elements	Bromine, Lithium, Silicon, Tin, Titanium.

- **Losee classification (1974) according to trace elements concentration in the enamel as shown in Table (2.3)**

Table (2.3): Classification of the trace elements according to their concentration in the enamel.[13]

a.	Elements found in Enamel at a median concentration above 10 µg/g dry wt.	Examples: F, Mg, Zn, Sr .
b.	Elements found in dental enamel at a median concentration between 1µg/g and 10 µg/g dry wt.	Examples: B, Al, Cr, Ba
c.	Elements found In dental enamel at a median concentration between 0.1µg/g and 1 µg/g	Examples: Mn, Cu, Se, Sn.

- **Classifications of trace element according to their relation to dental caries:**

(i) Navia, 1970 classified the trace elements as shown in Table (2.4):

Table (2.4): Navia classification.[40]

Cariostatic elements.	F, P
Slightly cariostatic elements.	Mo, V, Cu, Sr, B, Li, Au
Elements without influence on caries.	Ba, Al, Ni, Fe, Pd, Ti
Elements supporting caries.	Se, Mg, Cd, Pb, Pt, Si
Uncertain elements.	Br, Be, Co, Mn, Sn, Zn, and I.

(ii) Shashikiran, 2007 classified the trace elements as shown in Table (2.5):

Table (2.5): Shashikiran classification.[41]

Cariostatic elements.	F, P.
Mildly cariostatic.	Mo, V, Cu, Sr, B, Li, Au
Doubtful.	Be, Co, Mn, Sn, Zn, Br, I.
Caries inert.	Ba, Al, Ni, Fe, Pd, Ti.
Caries promoting.	Se, Mg, Cd, Pt, Pb, Si.

2.4. Tracing the element in the dental enamel:

Several studies were conducted to analyze the composition of human teeth and the researchers found that dental tissues contain a large number of minor inorganic constituents and trace elements in a varying quantity such as fluoride (F), strontium (Sr), vanadium (V), molybdenum (Mo), zinc (Zn), copper (Cu), manganese (Mn) and lead (Pb) in addition to the major components.[31,42] Furthermore, Armstrong and Brekhus, 1937, reported in their study that the composition of dental enamel varied from one individual to another and were not identical.[43] Brudevold *et al.,* 1967 attributed these variations to the different concentrations of trace elements that, were incorporated in the human dental enamel from the environment during and after the mineralization and maturation period of the tooth.[44]

Losee *et al.,* 1974 estimated 35 trace elements in the enamel of 28 sound bicuspid teeth collected from patients less than 20 years. The analysis was carried out for 66 minor inorganic elements of the whole enamel samples obtained from 24 communities located in 16 states in the United States. The communities were selected based on their geographic location, the chemical composition of their water supply, and with different caries prevalence. By using the spark source mass spectroscopy technique each sample was analyzed separately. It was found that 35 elements were present in enamel in detectable and quantitatable concentrations but the other 31 elements, if present, were below the detectable limits of the technique used in their study. Minor elements occurring in the greatest concentration (> 10 μg/g dry wt) were F, Mg, S, Cl, K, Zn and Sr. Others present in moderate concentration were Al, B, Cr, Fe, Mo, Ba and Pb in range of (1– 10 μg/g); and Li, Mn, Cu, Se, Br, Rb, Nb, Ag, Cd, Sn, Sb in a low concentration (0.1– 1.0 μg/g); Ti, V, Y, Zr, I, Cs, Ce, Pr, Nd and Bi were found at very low concentrations less than < 0.1μg/g. while 31 other elements could not be detected So, the authors concluded that at least 41 elements of the Periodic Table were incorporated into developing dental enamel.(13) Patel and Brown, 1975 reported that the chemical composition of pulverized dental enamel by chemical analysis according to Orban is a 2.3 % of water, 1.7 % of organic material and a 96 % of ashes, Regarding to the ash composition, is such that in a hundred grams of it, it is find: 36.4 Ca, 17.4 P, 2.7 Co_2, 0.4 Mg, 0.66 Na, 0.03 K, 0.01 F and 0.23 Cl , Beside, there were reported Silver (Ag), Strontium (Sr), Barium (Ba) , Chromium (Cr), Manganese (Mn), Vanadium (V), Aluminum (Al), Lithium (Li) and Selenium (Se) minimum amounts.(45)

However, Lakomaa and Rytömaa, 1977 evaluated the concentration of 12 different elements in primary and permanents teeth which were collected from six different localities in Finland. Na, Cl, Al, Mn, Ca, and P were determined by neutron activation analysis, K, Mg, Zn, Cu, and Fe by the atomic absorption method and F with the fluoride-specific electrode. They found that most of the elements were present in higher concentrations in the enamel than in the dentin except Mg which was higher in the dentin. Comparing to the permanent teeth, the primary teeth contained more K and Mn in enamel and K and Mg in dentin, but less Na and Zn in enamel.(46) Lane and Peach, 1997 measured the concentration of trace elements for dental enamel in 86 healthy human teeth, which were collected from Oxford shire in the United Kingdom, and Cornwall by using Particle-Induced X-ray Emission (PIXE). The authors detected the presence of these elements in dental enamel: K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Sr, Pb, and Hg. The concentrations of Fe and Cu were found to be lower in the teeth from female donors, and they attributed that to the continued burden of blood loss during menstruation.(47)

Another study was carried out by Reitznerová *et al.*, 2000 to investigate the concentration of seven elements (Cu, Fe, Mg, Mn, Pb, Sr and Zn) in the whole enamel thickness which was 200 µm. depth of extracted sound teeth of a population under and over 20 years and compared that with the first enamel surface layer which was 50 μm. in depth, by using Flame Atomic Absorption Spectroscopy (FAAS), Electro-Thermal Atomization-Atomic Absorption Spectroscopy (ETA-AAS), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma Mass spectrometry (ICP-MS). The results showed high concentrations of all microelements in the whole enamel thickness and that present in first surface layer. With exception of Sr and Mg, all elements showed significantly higher concentrations in the first layer than in whole enamel thickness and higher concentrations were present in the teeth of individuals over 20 years. The results were attributed to the difference in the concentration gradient which resulted from the interaction between saliva and teeth. The Cu, Fe, Mn, Pb and Zn concentrations in four layers of erupted and non-erupted teeth decreased while Mg and Sr concentrations increased toward the enamel-dentine junction. The concentrations of most elements were almost constant as they approached the 150 μm of layer depth.[48]

Also, Brown *et al.*, 2004 in his study proved that among different ethnic groups, there was a lot of variation in the concentrations of trace elements in dental enamel.[3] Amr and Helal, 2010 used Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to compare the trace element contents of primary to that of the permanent teeth. The samples were collected from 64 children and 112 permanent teeth from 40- 60 years old adults. Twenty elements were detected in this study. In comparison to primary teeth, permanent teeth contained significantly higher concentrations of Na, Mg, Al, Fe, Ni, Cu, Sr, Cd, Ba, Pb and U and significantly lower concentrations of Mn, Co, As, Se, Mo and Bi.[49]

2.5. Role of trace element in Dental caries:

Dental caries is considered as one of the most common preventable childhood diseases; people throughout their lifetime are susceptible to the disease and it is continues to be a significant dental disease and a major public health concern in children and adults around the world. (50) It is believed to be the primary cause of dental pain and tooth loss. (51)

2.5.1. Definition of dental caries:

Dental caries is an infectious microbiological disease that resulted in localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates.(52)

2.5.2. How dose caries occur?

Dental caries results from interactions over time between lactic and acetic acids produced by bacterial metabolic activities, and other host factors such as teeth and saliva factors.(51) It have been clarified that the initiation of dental caries is attributed to the ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms according to PH fluctuation in dental plaque.(53) Once the acid produced by metabolic process of the bacteria in dental plaque reaches to pH values below the critical value of 5.5 demineralization of tooth tissues occurred.(54)

Demineralization process is a dynamic phenomenon depends on diffusion of the calcium, phosphate, and carbonate out of the hydroxyapatite crystals of tooth structure.(55) If this cycle is continued, it will end up with an undermine tooth surface followed by breaking down of surface layer and a physical cavity will eventually take place.(56) Fortunately, demineralization can be reversed in its early stages through the deposition of calcium, phosphate, and fluoride.(57)

The fluoride acts as a catalyst for the diffusion of calcium and phosphate into demineralized tooth surface and rebuilt crystalline in the lesion forming fluoridated hydroxyapatite and fluorapatite which are much more acid-resistant than that of the original structure.[57,58] The importance of the trace elements in the prevention and reduction of dental caries has not yet been established fully[43]. However, various studies had been performed to find the link between trace elements and dental caries in human teeth.[5,14,15,41,59-61] The changes in the density of both macro and microelements in the tooth structure layers due to some environmental and genetic impacts produced some effects on human dental health. [62] It was found that the dissolution resistant of the tooth structure affected by the amount of F and ions such as Sn and Pb that form highly insoluble compounds, which make enamel more resistant to acid attack, whereas enamel with a high content of both Mg and carbonates is relatively susceptible to dissolution, and consequently the tooth will be more susceptible to dental caries.[40]

Barmes *et al*., 1970 studied the etiology of dental caries in Papua and New Guinea and they found that there was an inverse association between the development of dental caries and the presence of elements such as strontium, barium, potassium, calcium, and lithium. Also, they found that there was a direct association between the presence of chromium, zinc, selenium, lead, and copper with the development of new carious lesions.[63]

Curzon and Losee, 1978 performed a study to find out concentrations of 30 trace elements in the whole enamel of 83 caries-free teeth obtained from patients younger than 20 years of age. The samples were collected from a high-caries area (Oregon) and a low-caries area (California), and they used spark source mass spectrometry in the analysis of their samples. They found that copper was strongly associated with a high prevalence of caries and, to a lesser extent, aluminum, sulfur, titanium, chromium, nickel, silver, and tin. High concentrations of strontium in enamel associated with a low prevalence of caries.[15]

However, Shashikiran *et al*., 2007 carried out an in- vitro study to analyzing a 40 sound and carious primary and permanent teeth that extracted from children and adolescents of Davangere city to compare the concentration of eighteen trace element (F, Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se, Pb, Mo, and V) in the enamel of sound and carious primary and permanent teeth. The trace elements were estimated by using atomic absorption spectrophotometer; the authors reported that the concentrations of F, Sr, and K were significantly higher in the sound enamel of permanent teeth than in the sound enamel of primary teeth. The concentrations of F, Sr, K, Al, and Fe were significantly higher in the sound enamel of permanent teeth than in the carious enamel of permanent teeth. The concentrations of F, K, and Si were significantly higher in the sound enamel of primary teeth than in the carious enamel of primary teeth.(41) Riyat and Sharma, 2009 reported in their study that the Strontium was the only element found to be present in a significantly lower amount in carious teeth, thus they were strongly suggested that this element plays a role in the prevention of caries, and the presences of a greater amount of boron, manganese, molybdenum and fluorine in caries teeth indicated their role in predisposing or on causing dental caries.(61)

Ortiz *et al*., 2014 compared the distribution of chemical elements among the carious and normal enamel layers on teenagers, using Energy Dispersive X-Ray Spectrometer (EDS Rx) on 30 premolars of a teenager, 14 carious enamel layers and 16 normal enamel layers. They found that within the same tooth the macro and microelements differed in composition and varied from the external to the internal enamel layers between the carious and the sound premolars. However, the deficiency or excess of these elements in the enamel layers determines the degree of susceptibility to carious and other dental diseases. The results showed that the macroelements C, Ca, P and the microelements Al, Cl, Mg, Na were significantly different while, the macro O and the microelements In, Si, W, S were not significantly different among the carious and normal enamel layers. Moreover, the microelements Sb, Ba, Br, I, Ir, K, Pt, Sc, Sr, Sn and Yb were absent in carious enamel layers and present in normal enamel layers. They concluded that excess or deficiency of macro and microelements in enamel carious lesion were responsible for recurrent caries and dental diseases.[62]

More indeed, Peedikayil *et al.*, 2014 estimated the concentration of manganese, strontium and zinc in dental enamel by using inductively coupled Plasma atomic emission spectroscopy (ICP-AES), on a sample of 80 normal and carious deciduous molar teeth collected from children between the age group of 7-13 years. This study showed that sound enamel contains more strontium when compared to carious enamel while, manganese and zinc were more in carious enamel compared to the normal enamel. The concentrations of elements manganese, strontium and zinc did not vary with the sex of the individual.[64]

2.5.3. Role of the trace elements in enamel caries.

Trace element action on dental caries is not completely clear but according to Nizel and Papas, 1989 it was found that the possible mechanism could be by :

- ❖ Altering the resistance of the tooth itself by changing the size of the enamel crystals available to acid were a group of smaller crystals have a greater surface area and therefore, are more exposed to acid solubility than a group of larger crystals in enamel rods of similar size
- ❖ Modifying the local environment at the plaque-tooth enamel interface where they may inhibit or promote the growth of caries-producing bacteria.[65]

- **Role of Fluoride (F):**

Fluoride (F) is an essential part of the organized matrix in hard tissues such as teeth and bones and it is found in the form of fluorapatite.(66) The fluoride need of the body is met by drinking water, food, and tea.(67) The relationship between environmental F and human health has been investigated over 100 years by researchers from a wide variety of disciplines.(68) In the last century, scientific knowledge had been increased by several studies about the etiology and pathogenesis of dental caries and the deepening of evidence regarding the dynamic process of demineralization and remineralization has led to a consensus that taking primary preventive measures such as F applications associated with daily regular oral hygiene habits can stop or reverse dental destruction due to bacterial action.(69,70) The most well-known function of F is the prevention of tooth caries by making the enamel more resistant to the action of acids and accelerates the buildup of healthy minerals in the enamel, which lead to slowing the occurrence of decay.(71)

The mineral phase of the dental hard tissues is not pure hydroxyapatite (HAP = Ca_{10} $(PO4)_6$ OH_2), but rather is a calcium-deficient biomaterial in which numerous other ions are incorporated. Building of hydrogen phosphate, carbonate and magnesium ions into the HAP lattice leads to a less stable, more soluble apatite. In healthy human enamel, fluorhydroxyapatite (FHAP) or fluorapatite (FAP) are present in addition to HAP, although, in the outermost enamel layer, less than an average of 5% of the OH groups of HAP are replaced by F, this percentage drops further at a depth of 50 μm.(56) Fluorapatite is chemically stable but when pH in the oral cavity falls F is released. It is believed that fluoride's anti-caries effect is due to its ability to partly transform the carbonated hydroxyapatite (HA) in enamel to thermodynamically more stable fluorapatite (FA) or fluorhydroxyapatite (FHA). During the caries process, the

F is released, which makes changes in the dynamics of the demineralization/remineralization process on the tooth surface.[57]

Robinson *et al*., 1977 observed an interesting finding in early carious lesions of the enamel, whether from high or low F areas, contain more than double the F concentration of sound enamel from the same tooth. So, the combination of the fluoride to the enamel in early lesions can inhibit the progress of caries.[72] Machiulskiene *et al*., 1998 concluded that in children younger than 6 years, F is incorporated into the enamel of permanent teeth making the teeth more resistant to the action of bacterial and acids from foods, and in some cases, F can stop even already started tooth decay.[73]

On the other hand, the presence of high F concentration during calcification process of the teeth can lead to a kind of enamel hypoplasia known as dental fluorosis which clinically can vary from small white opacities in the enamel to severe mottling of the enamel structure with increasing severity.[74]
Based on continues researches done by Dean during the 1940s he recognize that up taking of optimal amounts of F can protect against the development of tooth caries without stain formation.[75,76] Then, it was suggested that the an optimal level of 0.7 -1.2 ppm of F should be added to the public drinking water supplies in the U.S., 1992.[77] Nowadays, most other developed countries and many people in developing countries are exposed to drinking water with natural F or at least get some F exposure through the use of toothpaste, mouth rinse, pediatric supplements, and various dental materials.[78]

Dedhiya *et al.*, 1974 found that F has a synergistic action with some elements like Sr and this interaction helps in reducing enamel dissolution.[79]
In a study done by Curzon and Crocker, 1978 in which the total enamel samples were taken from 451 teeth and chemically analyzed to estimate 30 trace elements, by spark source mass spectrometry, demonstrated that the presence of high F concentrations in whole enamel samples were associated with low

caries.[14] Shashikiran *et al.*, 2007 estimated and compared the trace element concentrations in sound and carious enamel of primary and permanent teeth the concentrations of F was significantly higher in sound enamel of permanent teeth than in carious enamel of permanent teeth. So they consider F as a cariostatic element.[41]

On the contrary Riyat and Sharma 2009 reported that the presences of a greater amount of F in caries teeth indicates their role in predisposing or can causing dental caries.[61]

- **Role of Strontium (Sr) :**

Strontium (Sr) is an element found everywhere in the environment, and it is present in all living organisms. Sr resembles calcium element in its properties, and it is taken up, and it is preferably implanted into the bone. Sr may have both beneficial and deleterious effects on humans health according to the consumed amount. [80] Steadman and Brudevol, 1958 stated that Sr is readily taken up by dental structures where it is deposited primarily before eruption and during tooth calcification, but posteruptively, additional strontium is laid down in the secondary dentin and cementum formed. It follows that children are most likely to have appreciable amounts of Sr.[81] While, Losee and Cutress 1974 found that Sr uptake occurs during tooth formation and there is no change in the strontium content of the tooth after the eruption.[14] On the other hand, Derise and Ritchey, 1974 found that Sr content of teeth was significantly greater in younger than in older age groups and the Sr content of teeth gradually reduced with age.[66]

Curzon *et al*., 1970 examined caries of 251 children aged 12 to 14, from two Ohio towns that use water supplies with high contents of boron and Sr showed a mean DMFS score of 3.56 compared to a score of 5.54 in a control group of 338 children. These findings suggested that the significantly lower caries prevalence in the former group was related to the boron and Sr content of the water rather than a 0.2 ppm difference in the fluorine level.[83] Dedhiya *et al*., 1974 in their vitro study showed a synergistic action between Sr and F in reducing enamel dissolution during the initiation of the caries process.[76] Curzon and Losee, 1977 stated that high Sr content was responsible for low caries incidence in human teeth.[84]

According to Curzon, 1985 apatites with Sr settlement are more difficult to remove from enamel compared to pure calcium component and it is stated that solution of enamel remineralized without Sr is more difficult than enamel remineralized with Sr. For this reason, it is believed that Sr adds resistance to hydroxyapatites against the dissolution. Sr settlement tooth enamel makes apatite crystal more resistant to caries due to the hetero-ionic change of Ca, so the resistance of apatite crystals against demineralization which occurs as a result of acid attacks is increased.[85] Which was supported by the later study of Vrbic *et al*., 1987, where they postulated that the Sr concentration is about constant in the surface and subsurface enamel. They further stated that administration of Sr salts before and after weaning could reduce the caries formation due to the possibility of substitution of Sr for calcium in the apatite lattice and the adsorption of calcium by the enamel surface.[17] Riyat and Sharma, 2009 postulated that the Sr was the only element found to be present in a significantly lower amount in carious teeth, thus strongly suggesting its role in caries prevention [61]. Ortiz *et al*., 2014 compared the distribution of chemical elements among the carious and normal enamel layers on teenagers, using an energy dispersive X-ray spectrometer (EDS Rx). Sr was absent in carious enamel layers and present in normal enamel layers.[62]

- **Role of Vanadium (V):**

Vanadium (V) is present in the soil, water, air and food. It is also found in continental dust, sea aerosol, and volcanic emissions, and industrial sources such as oil refineries. Also, it was reported that sea products generally contain vanadium at a higher concentration than the meat of land animals.[86] Daily vanadium uptake was reported in the range of 0.01–0.02 mg.[87] In experiments with V, a few animal studies were done to assess the role of vanadium in the development of dental caries.[88,89]

Geyer, 1953 found that when vanadium was given to hamsters after providing them with a cariogenic diet irrespective of the route, oral or parenteral, it promoted a higher degree of protection against dental caries.[88] Tempestini and Pappalardo, 1960 postulated in their study which involved combined administration of fluorine and vanadium in rats was more effective in reducing dental caries than the administration of fluorine alone.[90]

The findings of Tank and Storvick, 1960 were also interesting because they were able to show a reduction in caries prevalence in both deciduous and permanent dentitions with increasing concentrations of V in drinking waters. [18] However, Shaw and Griffiths, 1961 reported that there was no significant effect by administration of V as vanadium pentoxide or vanadyl chloride in drinking water or in food on the caries prevalence.[91] Similarly, Muhle,1964 administrated vanadium in drinking water at increasing levels, from 10 to 40 ppm. and their results showed that there were no effects on caries scores.[92]

Buttner, 1963 considered vanadium as cariostatic when it is uptaken into enamel during tooth development , but post eruptive administration enhances caries activity.[93] Curzon and Cutress, 1978 suggested that vanadium might have a cariostatic effect in man but its effect was not very predominant because of the presence of Se and F.[14] Curzon, 1984 illuminated the mechanism of vanadium in reducing caries prevalence thought to inhibit acid production by streptococci and actinomyces.[94]

- **Role of Molybdenum (Mo):**

Molybdenum is a chemical element with symbol Mo and atomic number 42 Molybdenum comprises 1.2 parts per million (ppm) of the Earth's crust by weight, but it is not find free in nature.[86] Hewat and Eastcott, 1962 in New Zealand, which was one of the more famous studies done to evaluate the role of trace elements in dental caries as they found that children consuming vegetables grown in the soil of the raised lagoon formed because of the earthquake that had taken place at Napier, had less caries prevalence as compared to the children in the nearby town of Hastings - an area which has the same water supply as Napier. Upon investigation, it was found that the trace element composition of the soil in these areas differed considerably with soil of Napier showing high concentrations of molybdenum as opposed to the soil of the town Hastings. Consequently, they proved that molybdenum has a cariostatic effect on teeth.[95] Adler and Straub, 1963 reported in a study done on children living in Hungary, that less prevalence of dental caries was noted in the area where there were traces of molybdenum found in water as compared to children from the area where there was no molybdenum in water.[96] In other study done by Davies and Anderson, 1987 found that molybdenum has been associated with reduced caries prevalence whereas selenium and lead appear to have adverse effects.[59]

- **Role of Copper (cu):**

Copper plays an essential role in our metabolism as it is involved in the functions of several critical enzymes.[97] Recommended daily copper level is 340 µg /day for 1–3 ages, 440 µg/day for 4–8 ages, 700 µg /day for 9–13 ages, 890 µg/day for 14–18 ages, 900 µg /day for males and females above 18 years of age, and 1000 µg /day for pregnant and 1300 mcg/day for nursing women.[98] Copper is present in oysters, sea animals with shell, whole grains, hazelnuts, potatoes, greeneries with dark leaves, dried fruits and animal products such as kidneys and livers.[99] Navia, 1970 listed copper as mildly cariostatic element.[40] Conversely, Barmes *et al*., 1970 studied the etiology of dental caries in Papua and New Guinea and they found that there was a direct association between copper with the development of new carious lesions.[63]

Lakomaa and Rytomaa, 1977 also reported that decayed teeth contained higher level of copper comparing to healthy teeth a result which was attributed to the existence of copper in water, food, soil or vegetables.[46] Similarly, Curzon and Losee, 1978 a reported that higher levels of copper have been found in carious teeth as compared to sound teeth.[15] Yem *et al*., 1991 performed a study to assess the role of trace elements in dental caries and they found the concentration of copper in normal teeth was low.[100]

According to Shetty and Kumara, 2012 copper was found to reduce the acidogenicity of plaque.[101] While Brudevold and Steadman, 1955 and Ashima *et al*., 1985 found in their studies that there was no correlation between the development of dental caries and the presence of copper in the enamel.[102,103]

- **Role of Aluminum (Al):**

Aluminium is the 13 element in the periodic table. One surprising fact about aluminium is that it is the most widespread metal on the Earth crust and the third most common chemical element on our planet after oxygen and silicon, and because of its reactivity, it is never found as free metal in nature, but it is always combined with other elements, such as oxygen, silicon, and fluorine.[86]

Kleber and Putt, 1985 and, Kleber and Putt, 1994 reported that aluminum considered as one of the element which may possess cariostatic properties. Their studies demonstrated that when Al solution is topically applied on the surface of both demineralized and sound enamel, it will firmly incorporate into the elements at the enamel surface and increased enamel resistance to acidic dissolution.[104,105] Tanaka, 2004 estimated the aluminum (Al) concentrations in the enamel and dentin of 314 human deciduous teeth to investigate the relationship between Al and dental caries. The teeth were divided into three groups: the sound tooth group, carious tooth group and filled tooth group. The carious tooth group was further classified into three groups depending on the stage of caries. The Al content was determined using graphite furnace atomic absorption spectrometry. It was discovered the Al concentrations in both enamel and dentin structures were unaffected by a gender, but they relayed on the tooth type. The Al concentration was significantly higher in the enamel and dentin of sound tooth group than in the other two groups. Furthermore, the Al level in carious did not decrease with the advance of caries. These findings indicated that the deciduous teeth containing higher Al concentrations had less caries than the teeth with lower Al concentrations, and it was suggested that Al acts as a possible cariostatic agent by itself.[106]

Curzon and Losee, 1978 drew the attention that Al, and some other elements, are positively related to enamel caries, as their concentrations were found evidently higher in carious enamel than in intact enamel [15]. Amin *et al.*,2016 Used graphite furnace atomic absorption spectrophotometry to estimate the quantity of eight trace elements in a sample of powdered enamel from Forty extracted primary and permanent human dentitions and they found that the concentrations of Al, Mn, and Se were significantly higher in decayed than in normal enamel, suggesting a positive association of these three elements with caries.[107]

- **Role of Selenium (Se):**

Selenium is a vital trace element that is required for several cellular functions and it is an essential component of antioxidant enzymes in the human body, but its excessive amount is toxic.[108] Selenium is found in liver, kidneys, sea products, meat, grains, grain products, milk products, fruits, and vegetables. The recommended daily intake is 70 µg.[99]

Hadjimarkos and Bonhorst, 1959 and Tank and Storvick, 1960 found that the uptake of selenium by human enamel during development, although the amount taken up was low (0.43 to 1.60 ppm) seemed to have a cariogenic effect. Also, they indicated a direct relationship between susceptibility of dental caries and presence of selenium excreted in urine in children living in areas where selenium did not occur naturally.[109,18]

Buttner, 1963 and Bowen, 1972 had shown that selenium administered to animals during tooth formation increased the incidence of caries, although Bowen, 1972 found that 1 ppm. selenium administered to monkeys after tooth eruption had an anti-cariogenic effect.[93,110]

- **Role of Manganese (Mn):**

Manganese is present in food with highest concentration in peanuts and lowest concentration in milk products, meat, poultry, fish, and sea products. In addition, 10 % presents in coffee and tea of daily intake.[111]

Manganese is considered as an enzyme activator in the human body with a concentration varies from 0.3 to 2.9 µg manganese\g, and it is stored in the bones, livers, pancreas, and kidneys typically these organs have higher manganese concentrations than other tissues.[112] Among the 49 elements in enamel hydroxyapatite crystals; one of them is manganese, which is usually present in very slight percentages. The manganese concentrations in enamel are between 0.08 and 20 ppm, and it is higher at permanent dentition compared to primary dentition. [113] Various studies had shown that manganese had a positive relation prevalence of tooth decay.[5,59] Peediayil *at el*., 2014 estimated three microelements in enamel of primary teeth where manganese was one of them.

This study showed that manganese concentration was more in carious enamel compared to the normal enamel and its concentration did not vary in relation to the sex of individual.(64) Also, Yem *et al.*, 1991 studied the effect of trace elements on dental caries in human tooth, and they found that the concentration of Mn in normal teeth was low.(100) On the other hands, Watanabe et al., 2009 found that the level of Mn in the sound and carious teeth was similar with or without treatment.(114)

- **Role of Zinc (Zn):**

Zinc is an essential element for the function of the human body and its deficiency or excess may produce negative effect on human body. (115) Zinc also supports normal growth in pregnancy, childhood, and adolescence periods It plays an essential role in cell reproduction, differentiation, and metabolic activities and its deficiency can lead to increased susceptibility to infections which reflects the development of defects in the immune system.(116)

The role of zinc in the development of dental caries is controversial.(5) Yem *et al.*,1991 assessed the role of trace elements in dental caries, and they concluded that the concentration of Zn was significantly higher in caries teeth than in normal teeth.(100) Tirth *et al.*, 2009 analyzed the presence of various trace elements in dental caries human teeth by external pixe, and they reported that the Zn concentrations in carious parts of the tooth were considerably very low.(117) However, in a study conducted by Ückardes *et al.*, 2009 to determine the effect of oral zinc supplementation on oral health in low socioeconomic level primary school children. The results showed improvement in the gingival index and reduction in the development of dental caries post-Zn supplementation.(118)
Peediayil *et al.*, 2014 estimated six trace elements in sound and carious enamel of primary teeth by atomic emission spectrometry, carious enamel showed higher concentrations of zinc.(64)

Gallagher and Cutress, 1977 studied the role of 25 trace elements present in or dissolved in the outer enamel surface of teeth in caries attack or plaque metabolism. They found that Zn exhibited a major inhibitory effect on the metabolism of S. mutans. They suggested that zinc might play an important role in the process of post-eruptive mineralization and reducing the susceptibility of teeth to caries.(119) Another study proved that zinc released from mouthwash solutions and toothpastes can continue to exist in plaque and saliva for a long time.(120) Based on this fact, zinc is added to oral health products in order to control plaques, reduce halitosis and delay tartar development.(116)

- **Role of Lead (Pb):**

People may expos to lead and to its toxic effect through consummation of food and beverage contaminated with this toxic metal.(121) Needleman *et al*., 1972 reported in a study done in urban and suburban American children, that lead played a major role in the development of new carious lesions in deciduous teeth.(122)

Bowen, 2001 reported that lead is transferred to teeth through environment or nutrition ways, and enhanced the process of dental caries especially in teeth with higher incidence of enamel hypoplasia as compared to children who were unexposed to such element.(60) Qamar *et al*., 2017 lead has the ability to translocated with Ca at the HAP of teeth leading to down size the HAP crystals.(123)

- **Role of Iron (Fe):**

Iron is an essential trace element that is responsible for the biological activities for living organisms in human body, and it is stored in liver, spleen and bone marrow with average amount 1-3 gm. of iron in human body.(98) The oral manifestation of iron deficiency can be seen as angular cheilitis, atrophic glossitis, diffused oral mucosal atrophy, candida infections, oral premalignant lesions, and stomatitis.[33] It was reported that adding 2 mmol/L ferrous sulfate to acidic beverage reduced mineral loss and human enamel preserved the enamel surface microhardness. So, iron acts as preventive agent against dental caries.[124] Emilson and Krasse, 1972 reported that by addition of iron salts to the diet or drinking water in some groups, in which the iron compounds were administrated topically on hamsters, the caries reduction was noted but it was observed that its effect was depending upon which salt was used.[125] Also, Curzon and Crocker, 1978 investigated the trace elements in tooth enamel for their role in caries and it was found that the presence of Fe was associated with the low risk of detal caries.[14] Similarly, Shashikiran *et al*., 2007 found that the concentrations of Fe were significantly higher in sound enamel of permanent teeth than in carious enamel of permanent teeth.[41]

- **Role of Boron (B):**

Boron is shown to be an essential element for plants earlier in this century and now, it becomes also a necessary element for humans.[33] The World Health Organization (WHO) put boron within the essential elements category, and expressed the amount of boron taken is 0.44 g /day via air, 0.2-0.6 mg/day via drinking water and 1.2 mg/day via diet.[126]

Boron present in a high amounts in hazelnut butter, avocado, peanuts and other nuts with shells, while coffee and milk contain a low amount of boron.[127] Various studies proved that B deficiency causes inhibition of the synthesis of the active form of vitamin D, which known as Calcitriol ($1,25\text{-}(OH)_2\text{-}D_3$) and coincided that an ingest up to 2-6 mg. B one daily helps to prevent arthritis (ART), osteoporosis (OST), osteoarthritis, cervical cancer, prostate cancer and cardiovascular diseases in people older than 45 years.[128,129] B is distributed throughout the human body with the highest concentration in the bones and with average 25-85 ppm. in dental enamel.[62,64] Curzon *et al.*, 1970 suggested that high concentration of both strontium and boron in water may contribute to lowering caries activity.[83] While, Navia, 1972 considered B as mildly cariostatic element in its classification to the trace element and its relation to the dental caries.[40] On the other hand, Riyat and Sharma, 2009 analysed 35 inorganic elements in teeth and their relation to caries formation and they found that boron was to be significantly increased in carious teeth than non-carious teeth despite loss of minerals during cariogenesis.[61]

- **Role of Potassium (K):**

The name is achieved from the English word potash, while the chemical symbol K comes from *kalium*, the mediaeval Latin for potash, that may have derived from the Arabic word *quail*, meaning alkali.[86] Potassium can be found in vegetables, fruit, potatoes, meat, bread, milk and nuts.[130] It plays an important role in the physical fluid system of humans and it assists nerve functions while, its deficiency in the diet can lead to hypertension.[131] Potassium nitrate added to the component of many of toothpaste as a desensitizing agent.[132] Shashikiran *et al.*, 2007 found that concentrations of K were significantly higher in sound enamel of permanent teeth than in carious enamel of permanent teeth. Also, it was significantly higher in sound enamel of primary teeth than in carious enamel of primary teeth. This result indicated that K is non-cariougenic in both primary and permanent teeth.[41]

- **Role of Nickel (Ni):**

Nickel is a compound that present in the environment only at very low levels and humans may be exposed to nickel by breathing air, drinking water, eating food or smoking cigarettes or skin contact, tea, chocolate and fats are rich source of nickel and contain severely high quantities.[133] Small quantities of nickel are essential for the body, but when the uptake is too high, it can be danger to human health as some studies have shown that acute exposure of human body to nickel may cause health problems.[134] Although the biological function of nickel is still somewhat unclear in human body, some believed that it is involved in protein structure or function, and acts as a cofactor in the activation of certain enzymes related to the breakdown or utilization of glucose.[135]

Navia, 1970 suggested that Nickel is one of the trace elements which have no influence on dental caries.[40] Gallagher and Cutress in 1977 studied the effect of 25 trace elements present in or dissolved in the outer enamel surface of teeth in caries attack or plaque on the growth and fermentation activities of oral streptococci and actinomyces and metabolism. They found that Ni exhibited major inhibitory effects on the metabolism of S. mutans so Ni may conceded as a cariostatic element.[119] This hypothesis was supported by study performed by Yem *et al*., 1991 which was made on number of trace elements and their possible rules in dental caries. The results showed that the distributions of nickel in normal teeth was significantly higher than that in dental carious teeth which concluded that Ni had inverse association to dental caries.[100]

- **Role of Cobalt (Co):**

Cobalt is an essential trace element for the human body, where it is considered as the main constituent of cobalamin which the scientific name of vitamin B12, and also, it plays role in the formation of amino acids and neurotransmitters in human body.[136] The routes of its entry to human body are via orally through food consumption, inhalation, or by skin contact, where it binds with proteins in the bloodstream and get transported to the body tissues and cells.[97] It was reported that the cobalt deficiency is associated with disturbances in vitamin B12 synthesis. It might cause anemia and hypothyroidism, as well as increase the risk of developmental abnormalities and failure in infants.[137] Navia, 1970 put Co under category of uncertain elements in dental caries prosses.[40] Also, Shashikiran *et al*., 2007 classified Co as a doubtful element in caries proses.[41]

- **Role of Chromium (Cr):**

Although chromium presents in low amounts in human body, it plays a major role in regulation of body metabolism and increases muscle and tissue build-up.[137] Cr is found in egg yolks, whole-grain products, high-bran breakfast cereals, coffee, nuts, green beans, broccoli, meat, and brewer's yeast.[99] Losee et al., 1974 reported in their study that chromium was present in moderate concentration which was about 0.1-18mg/g dry wt. in dental enamel.[13]

Al-Jorrani and El-Sammarai, 2013 performed study to determine the concentration of selected major (Calcium and phosphorus) and trace elements (Ferrous iron, nickel, chromium and aluminium) in permanent teeth and enamel among a group of adolescent 25 girls with an age of 13-15 years old in relation to severity of dental caries. The study group consisted of patients referred by orthodontists for extractions of upper first premolars. They reported that Cr ions played a role in improving mineralization and crystallity of teeth.[138]

- **Role of Magnesium(Mg):**

Magnesium is present in the form of magnesium phosphate in dental, and it has a great influence on the anatomy and quality of dental hard tissues.[139] It was reported that this element is responsible for inhibition of the crystal growth by replacing calcium ions in hydroxyapatite.[140] It was pointed out that Mg may also affect the activity of the alkaline phosphatase which catalyses the formation of appropriate hydroxyapatite crystals and may inhibit the transformation of amorphous calcium phosphate to a crystalline form.[141] Navia ,1970 found that Mg has been shown to have a significant association with dental caries and considered as a caries promoting element.[40] Le Geros, 1984 reported that calcium can be replaced to some extent by magnesium in HAP , although this is thought to be limited, but its incorporation by the hydroxyapatite lattice increases the acid solubility of apatite mineral. [142]

- **Role of Silicon(Si):**

Silicon is the most abundant electropositive element on the Earth [86]. This element exists in all body tissues, but it shows highest concentration in bone, skin, hair, nails and arteries [143], and it is considered as one of the essential elements for bone formation and its deficiency in mammalian species results in depressed growth and skull deformities.[144] Silicates and silicate- based compounds are used materials in dentistry such as fillers like glass-ionomer cements, compomers, composites to improve their mechanical properties, also, silicates are used as a component of dental ceramics for instant for veneers, inlays , onlays and for full- ceramic crowns.[145]

Navia, 1970 considered Si as element that supporting tooth caries [40]. Cardenas *et al*., 2014 in study done on trace element concentrations and distribution in dental enamel by using the energy-dispersive X-ray spectroscopy technique they found 0.06 % of silicon ion (Si) was present in the entire enamel thickness, particularly in the inter-cuspid zone of the tooth.[146] Shashikiran *et al*., 2007 found that concentrations of Si was significantly higher in sound enamel of primary teeth than in carious enamel of primary teeth and on other hand, Si was significantly higher in carious enamel of permanent teeth than in sound enamel of permanent teeth. This could formulate Si as carious promoting element in permanent teeth and non cariogenic in primary teeth.[41]

2.6. Trace Elements Analysis Techniques:

2.6.1. Spectroscopic techniques for estimation of (Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se ,Pb, Mo, and V):

Various analytical methods had been applied for determination of trace elements in dental enamel. Atomic absorption spectrophotometry (AAS) and atomic emission spectroscopy (AES).[8,41,48,49,64] electrochemical methods [46], proton induced X-ray emission (PIXE) [147], and laser ablation ICP-MS (LA-ICP-MS).[148] Overall, it was found that atomic absorption/emission spectroscopy (ICP-AES) techniques also termed inductively coupled plasma optical emission spectrometry (ICP-OES) was proposed in 1960s and developed rapidly since 1970s. It is a quantitative multi-element measuring system that offers wide detection range of elements, good stability and reproducibility, low detection limit, and various sample introduction techniques.[147,149-151]

In our study the samples send to Metallurgical Research & Devlopment Institute (CMRDI) in Ministry of Scientific Research Center in Egypt and the estimation was done by (ICP-AES Optima 2000 DV Perkin Elmer).

2.6.2. Electrochemical techniques for estimation of F:

In the meantime, ion-selective electrodes (ISE) had been widely used for determining fluoride concentrations.[152] The potentiometry with ion-selective electrodes (ISE) is considered the method of more results accuracy [152]. Hence, the other techniques like flow injection analysis, gas chromatography, ion chromatography, capillary electrophoresis, flame atomic absorption spectrometry, and spectrophotometry, as well as inductively coupled plasma-mass spectrometry, these techniques are complicated and time-consuming and give an incomplete breakdown of the fluoride containing in the sample to be analysed as free ions.[154]

The samples of this study analysed by (HANNA\Hi 98402 ISE Meter Fluoride) in privet biochemical lab in Egypt.

2.7. Important of the study

Dental caries is still a major oral health problem in the world, affecting 60–90% of schoolchildren and the vast majority of adults.[155] Globally, it was estimated that 2.4 billion people suffer from caries of permanent teeth and 486 million children suffer from caries of primary teeth.[156]

An ongoing challenge for professionals and researchers regarding oral health is the control and the prevention of dental caries. Hence, the analysis of chemical element contents of the teeth can expand the knowledge of the etiology of dental diseases and it can be used for a diagnostic, therapeutic and preventive purposes.[79] So, the aim and the objective of this in vitro study were to estimate and compare the trace element concentrations in sound and carious enamel of primary and permanent teeth and finding the relation between these elements and prevalence of dental caries in children and adolescents in Benghazi city.

3.Aim of the study

The aim of the study was to estimate of some trace elements in sound and carious enamel of primary and permanent teeth of children in Benghazi and evaluation of correlation between the presence of caries and trace elements.

Objectives of the study

This study was designed to:

1. Estimate and compare the trace element concentrations including the fluoride in sound and carious enamel of primary and permanent teeth in samples of children from Benghazi, and finding their relation to dental caries.
2. Assess the presence of fluoride use in Libyan children.
3. Provide a basic line data and encourage for any future study.
4. Provide information for the health authority.

4.Materials and Methods:

In this an in-vitro study was carried on a sample of total 48 sound and carious primary and permanent teeth from patients living in Benghazi city where their age was between 7-20 years old for a purpose to estimate the concentration of 18 trace element in dental enamel and finding their relation to dental caries in human teeth.

4.1. Materials:

The materials used in this study shown in Table (4.1):

- Table (4.1): Materials used in the study.

Name of the product	Manufacturer	Uses
Nitric Acid 2%	Lab Alley	To produce a clear solution.
Doubl-Distilled Deionized Water	Vater X	Diluting the solution.
Standard Solutions	Prepared By An Expert Chemist	To make quality control to the device and create the calibrate curve to detect the concentration of each element to be analyzed.
(TISAB) A Total Ionic Strength Adjustment Buffer TISAB-II	(HI 4010-00)	Buffer solution added to adjust the total ionic strength, and release the F ions in the solution.
HCl	Lab Alley	Dissolving the enamel bower.
Sodium Acetate Solution 15%	Chemazone	Increase the PH of the solution, and get more free F.

4.1.1.Armamentariums:

The armamentariums used in the study are listed in Table (4.2):

Table (4.2) : Armamentariums used in the study.

Name	Manufacturer	Use
4 Soft bristle toothbrushes	Oral-B	To remove debris and blood.
2 Sharp clean dry spoon excavators.	Nordent #3,#4	To remove the soft dental caries.
Straight hand piece .	(micro-motor unit rx-mc-90)	Grinding the enamel.
2Cylindrical and 2 Conical Alpine Tooth Grinding Stones.	Ultradent	Grinding the enamel.
4 Transparent plastic boxes.	Dunya plastic	To prevent the resulting enamel dust from becoming airborne and lost.
4 Air-tight plastic bags.	Falcon	Containing the resulting enamel dust.
Dental napkins	Dento-premium	Placing the instrument on during preparation.
Volumetric flask	Pyrex	Mixing the teeth powder in the acid.
Stickers	Flamingo	Label the samples.
Black marker	Parmanet	Label the samples.
Phone camera version 10.0.03.88	Samsong sm-m30	Taking photos
Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)	ICP-OES Optima 2000 DV Perkin Elmer	Trace element analysis.
Fluoride ion -selective electrode (ISE)	HANNAL/ HI98402 ISE Meter Fluoride	Fluoride analysis.

4.2.Method:

Trace elements enter the human body coming from different sources such as food, water, air, etc., they can be incorporated in tooth enamel structure and they could affect the enamel resistant to dental caries.[99]

4.2.1. Study Design

An in-vitro experimental study was performed to assess the trace elements in 48 samples of an enamel powder which were collected from sound and carious permanent and deciduous teeth by using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Analysis and Fluoride ion selective electrode.[151-152]

4.2.2. Study sample:

Forty-eight extracted sound and carious primary and permanent teeth were collected from patients attended to different private and governmental dental clinics in Benghazi city according to the selection criteria used in the study.[41] The selection criteria were as follow:

Inclusion criteria

a) Sound primary incisors that had been extracted for reasons of pre- shedding mobility or over-retention in children with an age-group of 7-12 years.

b) Carious primary incisors that had been extracted for reasons of carious involvement, pre-shedding mobility or over-retention in children with an age-group of 7-12 years.

c) Sound premolars that had been extracted for orthodontic reasons in adolescents with an age-group of 14-20 years.

d) Carious premolars that had been extracted for carious destruction in adolescents with an age-group of 14-20 years.

e) The teeth were taken from patients borne and grow up in Benghazi city.

Exclusion criteria

a) Extracted teeth with developmental anomalies and fluorosis.

b) Teeth extracted from patients suffering from systemic diseases.

C) The extracted teeth had no previous restorations.

4.2.3. Consent form

Information sheet that explained the purpose of the project was given to the participants and signed written informed consents were obtained from all the subjects selected for the study (Appendix 1). All subjects were made aware that their extracted teeth were included in this investigation and could be used in other research studies.

4.2.4. Samples collection and preparation.

- All the collected teeth were cleaned thoroughly under running tap water using soft bristle toothbrushes to remove debris and blood.

- Then a sharp clean dry spoon excavator was used to remove the soft dental caries from each carious sample and the collected samples were left to dry overnight.

Figure 4.1: Removing caries by sharp clean dry spoon excavator and left the samples to dry overnight.

4.2.5. Sample grouping.

The samples were divided into 4 groups with 12 samples included in each group (n=12) according to the type of the teeth whether permanent or deciduous and the conditions of the teeth weather sound or caries according to the inclusion criteria of the study .

Each group was placed in sterile Petri dishes and labeled as following:

Group A: Included 12 sound primary incisors (n=12).

Group B: Included 12 carious primary incisors (n=12).

Group C: Included 12 sound permanent premolars (n=12).

Group D: Included 12 carious permanent premolars (n=12).

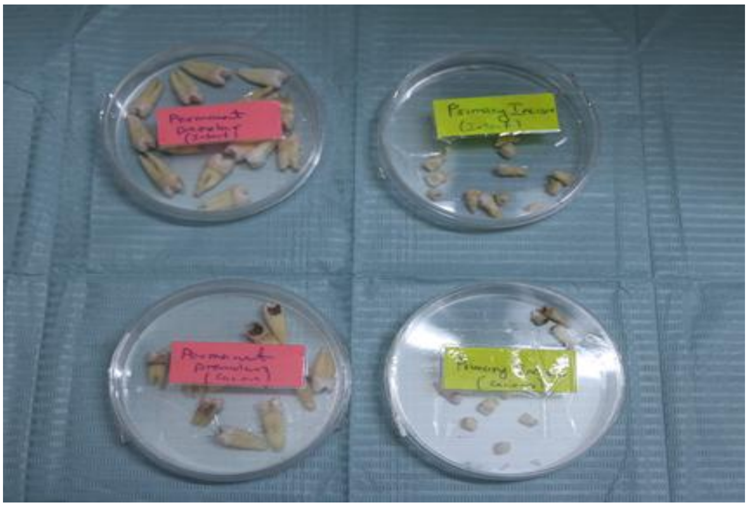


Figure 4.2: Teeth used in the research placed in petri dishes.

4.2.6. Sample grinding.

Mechanical grinding had been done by using a cylindrical alpine tooth grinding stone fitted to a straight hand piece (micro-motor unit Kavo, EWL K-11) at a fixed speed of 10,000 rpm in order to avoid excessive heat and the grinding process was limited to the tooth enamel.[8,41]

The whole grinding procedure was undertaken inside a transparent plastic box to prevent the resulting enamel dust from becoming airborne and lost, each group were mechanically ground using a new sterile bur and a different plastic box, to overcome the contamination between the groups.

Care was taken to confine the grinding within the enamel layer and without exposing dentine. The enamel powder collected from each plastic box was kept in sterile small air-tight plastic bag.[41]

Figure 4.3: Teeth grinding done by using a cylindrical alpine tooth grinding stone fitted to a straight hand piece (micro-motor unit Kavo, EWL K-11) inside a transparent plastic box.

Figure 4.4: Collected Enamel Powder in a plastic pages.

4.2.7. Preparation of the wet-ashed solution

Wet-ashing is a process of dissolving enamel powder in a suitable acid to produce a clear solution.

I. Preparation of solution for TEs analysis by ICP-OES:

- One gram of enamel dust from enamel sample A was taken in a volumetric flask and was wet-ashed in 2 ml of nitric acid to produce a clear solution.[46]

- The solution was further diluted by using double-distilled deionized water to make the volume 100 ml. So, the final solution contained nitric acid at a concentration of 1M (Molar solution), prepared solution was labeled as solution A.
- Enamel samples B, C, and D were similarly processed to make solutions B, C, and D, respectively.

- The standard solutions were prepared by an expert chemist in Central Metallurgical R & D Institute in Cairo city , the standard solutions contained about 1000 µgm/ml. of the particular element.

- The working standard solutions were prepared by suitable dilution of the standard solutions for each element to be analyzed.

II. Preparation of solution for F analysis by Fluorid SE meter:

- One gram of enamel powder from sample A was taken in a volumetric flask and was wet-ashed in concentrated HCl then, a 900 µL sodium acetate solution with concentration 15 % was added to each 100 µL of dissolved enamel samples to increase the pH of the solution, Then, 900 µL of this solution was taken and 100 µL of TISAB-II buffer solution (HI 4010-00) was added to adjust the total ionic strength, and release the F ions and hence increases the accuracy of the reading.

The same procedures were applied on samples B,C and D to make final solutions B, C, and D, respectively.

HANNA calibration standard solutions with F concentration : 1 ppm , 2 ppm, 10ppm,100ppm,1000ppm were prepared.

4.2.8. Instruments used in samples analysis

I. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Analysis.[11,157]

It is an optical instrument that detect the elements concentration by measuring the intensity of light in relation to the wavelength of each element.

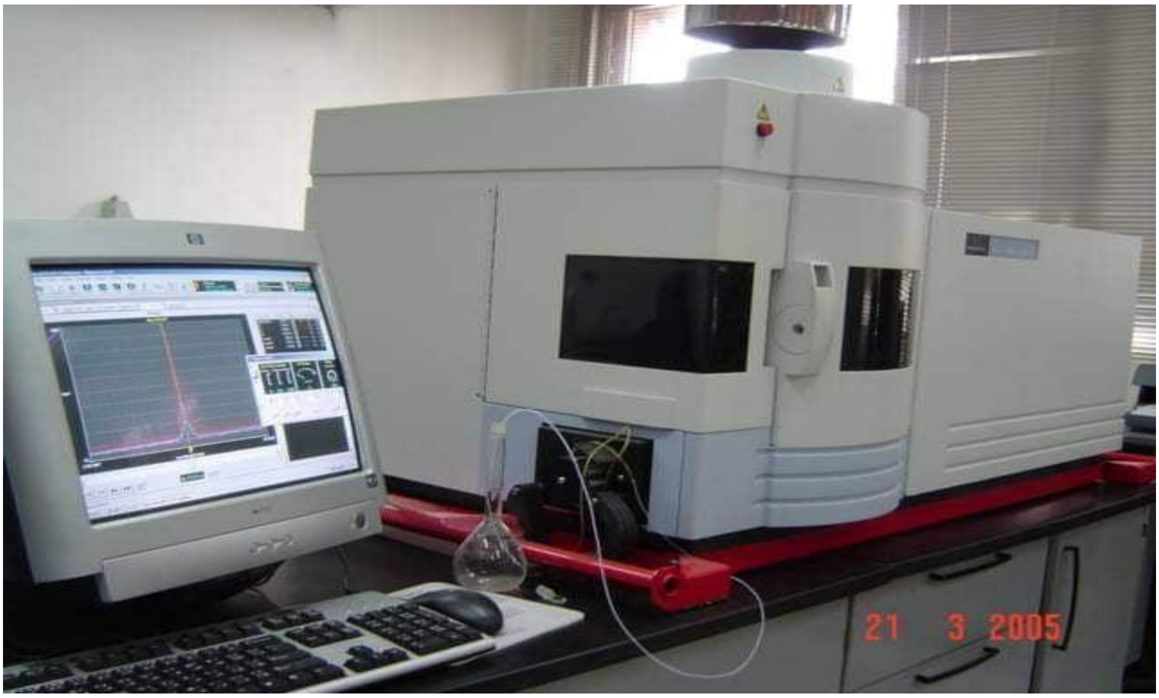


Figure 4.5: The Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) in the Central Metallurgical R & D Institute lap.

- **The principle of Spectrophotometer device:**

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry, Which uses the fact that when atoms and ions absorb energy electrons move from the ground state to an excited state and that is happen by using heat from an argon plasma as a source of this energy.

Basically, each atom is composed of a nucleus bordered by electrons. Every element has a specific number of electrons connected to its nucleus. The most stable orbital configuration of an atom is known as the "ground state. When plasma energy is applied to an atom in the analysis sample, a known quantity of energy with a given wavelength will be absorbed and an outer electron then promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will spontaneously return to the "ground state, then it releases emission rays (spectrum rays) so, the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the rays' intensity.[158]

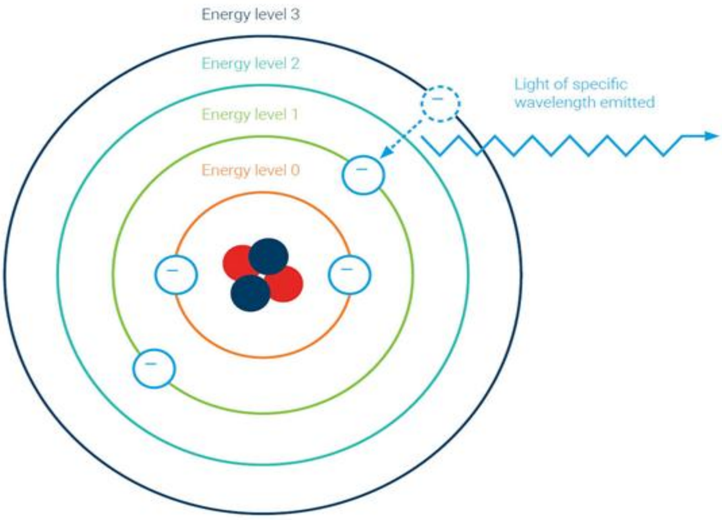


Figure 4.6: The diagram shows the energy levels in a lead (Pb) atom.[159]

In brief the ICP-OES instrument works by:

1. Using an argon plasma to give energy to exciting atoms or ions, then the atoms or ions will translate from grounded state to excited state.
2. when the electrons in the atoms or ions return to the ground state, or a lower energy state, the intensity of the light emitted Measured by the devise.
3. Calculating the concentration of specific elements in a solution, based on a calibration graph.

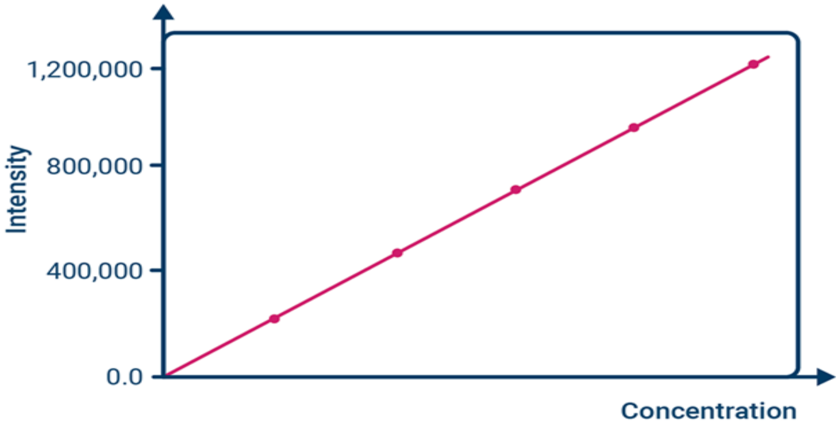


Figure 4.7: The calibration graph for Pb shows the linear relationship between the concentration on the X-axis and the emission intensity on the Y-axis.(158)

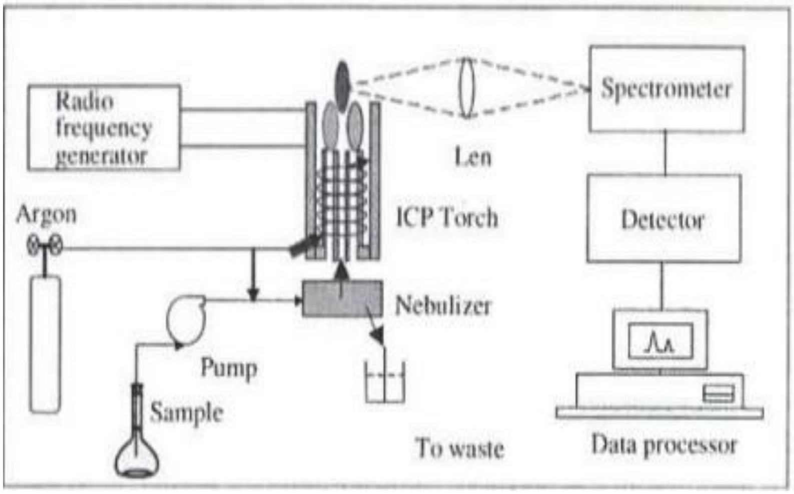


Figure 4.8: Schematic diagram of inductive coupled plasma-optic emission spectrometry (ICP-OES). (159)

II. Fluoride ion -selective electrode (ISE):

It is the most widely used method for the determination of fluoride in biological samples, where it is deemed simple to perform and has good precision and sensitivity.(160)

Figure 4.9: HANNAL/ HI98402 ISE Meter Fluoride.[161]

- **The principle of a fluoride ion selective electrode**

It is an electro-analytical method works based on the principle of potentiometry in a galvanic cell. It consists of a reference electrode, ion-selective membrane, and voltmeter. The inner surface of the membrane is in contact with the inner reference solution, and the outer surface is in contact with the sample solution containing the analyte as in Figure (4.10).

A potential develops across the membrane that depends on the difference in the concentration of fluoride ion on each side of the membrane.

This potential is measured with respect to a stable reference electrode having a constant potential, and a net charge is determined.

Since the concentration of the internal solution is fixed (standard solution), the potential developed across the membrane is related to the concentration of the analyte.[161]

The electrode first calibrated with calibration standard solutions with known F concentration then electrode potentials of standard solutions are measured and plotted on the linear axis against their concentrations on the log axis.[162]

The readings of fluoride levels the enamel solutions were calculated using the slope of the calibration curve [162] As showed in figure (4.11)

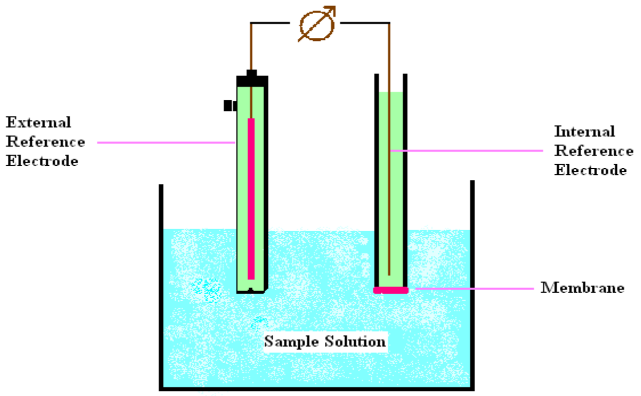


Figure 4.10: Electrochemical cell for making a potentiometric measurement with an ISE.[163]

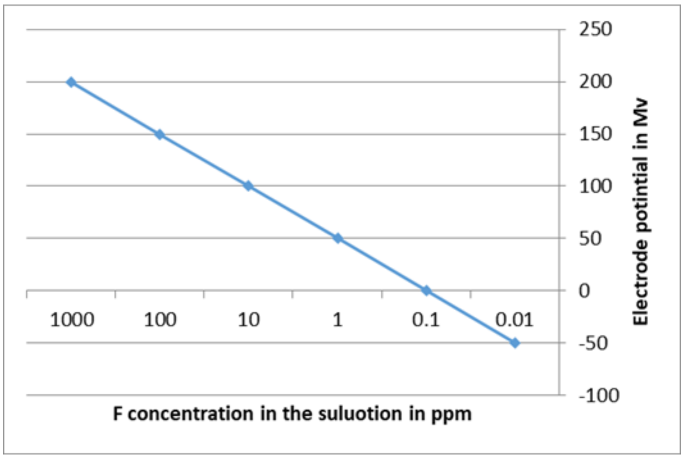


Figure 4.11: Example of fluoride calibration curve.[161]

4.2.9. Trace element analysis

I. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Analysis.

For analysis of (Al,Sr,B,Mn,V,Pb,Se,Zn,K,Si,Ni,Fe,Cu,Co,Cr,Mo and Mg) trace element the prepared solution containing the materials that are to be analyzed and the standard solutions (with the known concentrations of these elements) is introduced into (ICP-OES) and measuring the intensities of light at appropriate analytical line .The data curve determines the relationship between the intensity of light emitted at a specific wavelength and the concentration of the element in the solution for each element. An example of a linear calibration curve is demonstrated by (Fig. 4.11).

The concentration of the elements in the test solution was read from the calibration curve. Three readings values were recorded to each element in parts per million (ppm).

II. Fluoride Ion Selective Electrode:

The fluoride concentration was then determined using an ion selective electrode (HANNAL/ HI98402 ISE Meter Fluoride) shown in figure (4.9). The electrode was calibrated with the standard solutions first then, The readings of electrode potential were recorded with the ion meter at 15 min contact time after immersing the electrode into the continuously stirred solution at 25 °C. Rinse electrode tip with distilled water between readings.

The calibration curve was generated from the standard fluoride solutions ranging from 1 to 1000 ppm fluoride ions from its stock solution by plotting the observed electrode potential (mV) vs logarithm of F concentration (ppm) to obtain a straight line (Fig. 4.11) The three readings of fluoride levels in the enamel solutions of each ample were calculated using the slope of the calibration curve.

4.2.10. Statistical analysis.

The concentrations of the 18 trace elements, F, Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se ,Pb, Mo, and V were estimated in all enamel samples. Three readings were recorded for each element (in the same sample synchronously), the mean, standard deviation (SD), and range (mean ± 2 SD) were calculated. The values obtained were tabulated and the differences between the means in the four groups were compared using the nonparametric T- test.

5. Results

A total of 48 extracted sound as well as carious primary and permanent teeth were used in the study analysis to determine the trace elements in the enamel . The results of this study showed the presence of 14 trace elements (Al, Sr, B, Mn, V, F, K, Si, Ni, Fe, Cu, Co, Cr and Mg) in enamel of sound and carious primary and permanent teeth. The concentrations of Pb, Mo, Se and Zn were below 0.1 ppm (detection limit of the equipment = 0.1 ppm).The results were summarized in table 1,2,3 and 4.

5.1. Trace elements in sound and carious permanent teeth:

Table (5.1) shows the results obtained from the analysis of trace elements in enamel of sound and carious permanent teeth. The most common trace element found in both sound and carious permanent teeth was Sr following by K, F and Al. On the other hand, the least observed trace elements were Mg, Cr, Co & Cu. Other elements such as Pb, Se, Zn, and Mo were not detected in either sound or carious permanent teeth.

Table 5.1: Trace elements in sound and carious permanent teeth.

Permanent Sound teeth			Permanent carious teeth			
	Mean	S.D.		Mean	S.D.	P value
Al	80.38	2.31	**Al**	107.5	0.68	<0.001***
Sr	546.5	6.37	**Sr**	413.27	2.8	<0.001***
B	30.53	1	**B**	55.03	1.12	<0.001***
Mn	19.23	0.82	**Mn**	13.6	0.5	0.001**
V	4.7	0.4	**V**	1.38	0.56	0.001**
Pb	ND		**Pb**	ND		---
Se	ND		**Se**	ND		---
Zn	ND		**Zn**	ND		---
F	195.7	5.42	**F**	170.54	3.77	0.003**
K	243.33	21.03	**K**	244.67	28.75	0.951
Si	12.27	1.47	**Si**	18.37	1.66	0.009**
Ni	8.73	0.99	**Ni**	13.1	0.55	0.003**
Fe	2.9	0.42	**Fe**	8.23	0.46	<0.001***
Cu	3.43	0.7	**Cu**	4.5	0.75	0.146
Co	2.55	1.13	**Co**	2.7	0.78	0.891
Cr	3.67	0.45	**Cr**	3.17	0.57	0.299
Mo	ND		**Mo**	ND		-----
Mg	0.25	0.06	**Mg**	0.17	0.04	0.058

ND: Not detected.

S.D.: Stander deviation.

5.2. Trace elements in sound and carious primary teeth:

Table (5.2) shows the results obtained from the analysis of trace elements in enamel of sound and carious primary teeth. The most common trace element found in both sound and carious primary teeth was Sr, followed by K, F and B. On the other hand, the least observed trace elements were Mg, Cr, Co & Fe. Other elements such as Pb, Se, Zn, and Mg were not detected in either sound or carious permanent teeth. Mo in primary carious teeth was not detected.

Table 5.2: Trace elements in sound and carious primary teeth.

Primary sound Teeth			Primary carious Teeth			
Element	Mean	S.D.	Element	Mean	S.D.	P value
Al	19.61	0.62	**Al**	16.7	0.56	0.004**
Sr	171.83	1.04	**Sr**	192.7	5.13	0.002**
B	39.53	1.06	**B**	37.6	0.44	0.043*
Mn	39.4	0.75	**Mn**	2.77	1.19	<0.001***
V	12.61	0.19	**V**	9.03	0.35	<0.001***
Pb	ND		**Pb**	ND		-----
Se	ND		**Se**	ND		-----
Zn	ND		**Zn**	ND		-----
F	120.69		**F**	108.91	3.13	0.006**
K	143.2	25.04	**K**	144.37	5.01	0.941
Si	26.5	1.6	**Si**	15.73	2.8	0.004**
Ni	12.23	0.65	**Ni**	9.67	0.87	0.015*
Fe	6.83	0.65	**Fe**	6.33	0.6	0.384
Cu	8.2	0.7	**Cu**	8.37	1.7	0.883
Co	1.77	0.57	**Co**	2.31	0.55	0.467
Cr	1.5	0.36	**Cr**	0.67	0.25	0.03*
Mo	ND		**Mo**	ND		-----
Mg	0.12	0.006	**Mg**	ND		(NA)

ND: Not detected.

S.D.: Stander deviation.

5.3. Comparisons of trace elements in primary and permanent sound teeth:

By comparing the results in table (5.3) obtained from the analysis of trace elements in enamel of sound primary and permanent teeth a significant differences were observed in most of trace elements. Sound Primary teeth have significantly higher amount of B, Mn, V, Si, Fe, Ni & Cu ($p < 0.05$). Sound permanent teeth have higher amount of Al, Sr, F, K, Cr & Mg ($p < 0.05$). While no significant differences were found between sound primary and permanent teeth in amount of Co.

Table 5.3: Trace elements in primary and permanent sound teeth.

Primary Sound Teeth			Permanent Sound Teeth			
Element	Mean	S.D.	Element	Mean	S.D.	P value
Al	19.61	0.62	**Al**	80.38	0.68	<0.001***
Sr	171.83	1.04	**Sr**	546.5	2.8	<0.001***
B	39.53	1.06	**B**	30.53	1.12	0.001**
Mn	39.4	0.75	**Mn**	19.23	0.5	<0.001***
V	12.61	0.19	**V**	4.7	0.56	<0.001***
Pb	ND		**Pb**	ND		-----
Se	ND		**Se**	ND		-----
Zn	ND		**Zn**	ND		-----
F	120.69		**F**	195.7	3.77	<0.001***
K	143.2	25.04	**K**	243.33	28.75	0.010*
Si	26.5	1.6	**Si**	12.27	1.66	0.001**
Ni	12.23	0.65	**Ni**	8.73	0.55	0.002*
Fe	6.83	0.65	**Fe**	2.9	0.46	0.001**
Cu	8.2	0.7	**Cu**	3.43	0.75	0.001**
Co	1.77	0.57	**Co**	2.55	1.13	0.276
Cr	1.5	0.36	**Cr**	3.67	0.57	0.005**
Mo	ND		**Mo**	ND		
Mg	0.12	0.006	**Mg**	0.25	0.04	0.003**

ND: Not detected.

S.D.: Stander deviation.

5.4. Comparisons of trace elements in primary and permanent carious teeth:

Table (5.4) compares the results obtained from the analysis of trace elements in enamel of carious primary and permanent teeth. While significant differences were observed in most of trace elements, no significant differences were found between carious primary and permanent teeth in amount of Co & Si . Carious primary teeth have higher amount of V & Cu ($p < 0.05$). Carious permanent teeth. have significantly higher amount of Al, Sr, B, Mn, F, K, Ni, Fe & Cr ($p < 0.05$).

Table 5.4: Trace elements in primary and permanent carious teeth

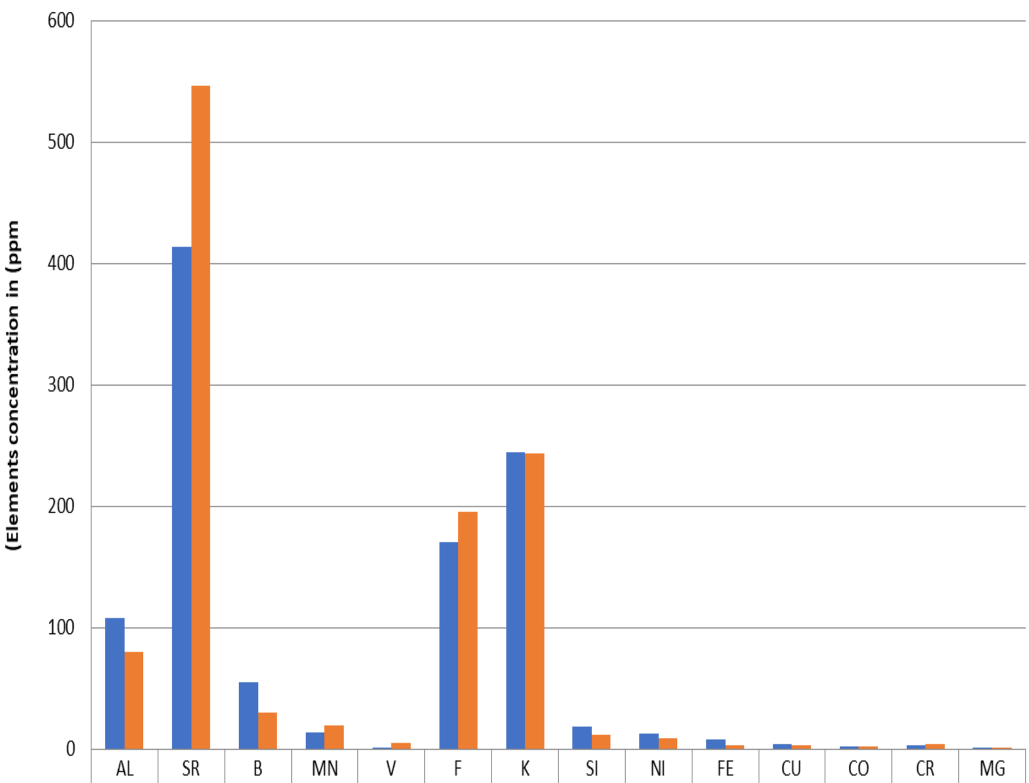
Primary Carious Teeth			**Permanent Carious Teeth**			
Element	Mean	S.D.	Element	Mean	S.D.	P value
Al	16.7	0.56	**Al**	107.5	2.31	<0.001***
Sr	192.7	5.13	**Sr**	413.27	6.37	<0.001***
B	37.6	0.44	**B**	55.03	1	<0.001**
Mn	2.77	1.19	**Mn**	13.6	0.82	<0.001***
V	9.03	0.35	**V**	1.38	0.4	<0.001***
Pb	ND		**Pb**	ND		
Se	ND		**Se**	ND		
Zn	ND		**Zn**	ND		
F	108.91	3.13	**F**	170.54	5.42	<0.001***
K	144.37	5.01	**K**	244.67	21.03	0.001**
Si	15.73	2.8	**Si**	18.37	1.47	0.119
Ni	9.67	0.87	**Ni**	13.1	0.99	0.011*
Fe	6.33	0.6	**Fe**	8.23	0.42	0.011**
Cu	8.37	1.7	**Cu**	4.5	0.7	0.022*
Co	2.31	0.55	**Co**	2.7	0.45	0.491
Cr	0.67	0.25	Cr	3.17		0.001**
Mo	ND		Mo	ND		
Mg	ND		Mg	0.17	0.06	(NA)

ND: Not detected.

S.D.: Stander deviation.

5.5. Comparisons of trace elements in primary and permeant teeth:

Figure (5.1) describe the mean and standard deviation (SD) of trace elements detected in the samples.



	AL	SR	B	MN	V	F	K	SI	NI	FE	CU	CO	CR	MG
Carious	107.5	413.27	55.03	13.6	1.38	170.54	244.67	18.37	13.1	8.23	4.5	2.7	3.17	0.17
Sound	80.38	546.5	30.53	19.23	4.7	195.7	243.33	12.27	8.73	2.9	3.43	2.55	3.67	0.25

	P value
AL	<0.001***
SR	<0.001***
B	<0.001***
MN	0.001**
V	0.001**
F	0.003**
K	0.951
SI	0.009**
NI	0.003***
FE	<0.001***
CU	0.146
CO	0.891
CR	0.299
MG	0.058

Figure 5 .1: Comparisons of trace element in enamel of sound and carious permanent teeth.

Figure (5.1) depicts comparison of trace elements in sound and carious permanent teeth. While significant differences were observed in most of trace elements, no significant differences were found between sound and carious permanent teeth in amount of K, Cu, Co, Cr & Mg . Carious permanent teeth have significantly higher amount of Al, B, Si, Ni & Fe ($p < 0.05$). Sound permanent teeth have higher amount of Sr, Mn, V& F ($p < 0.05$).

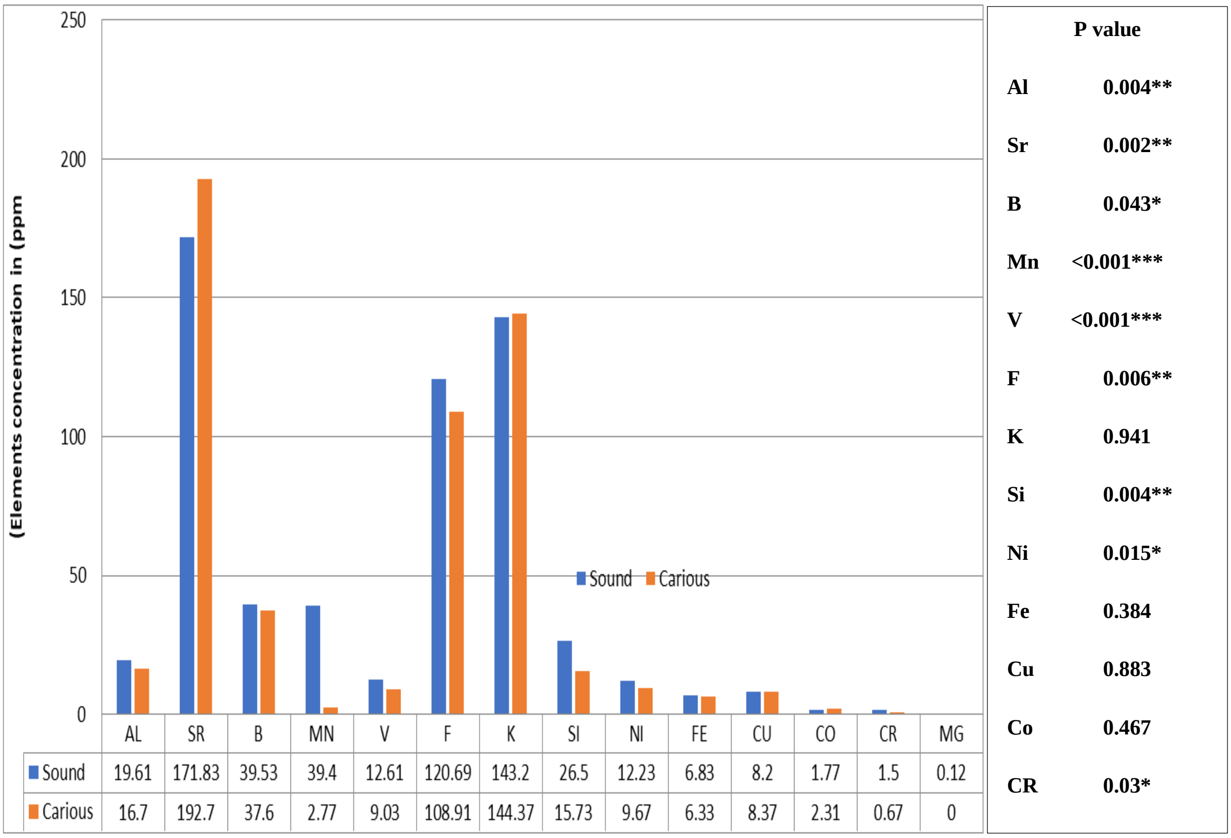
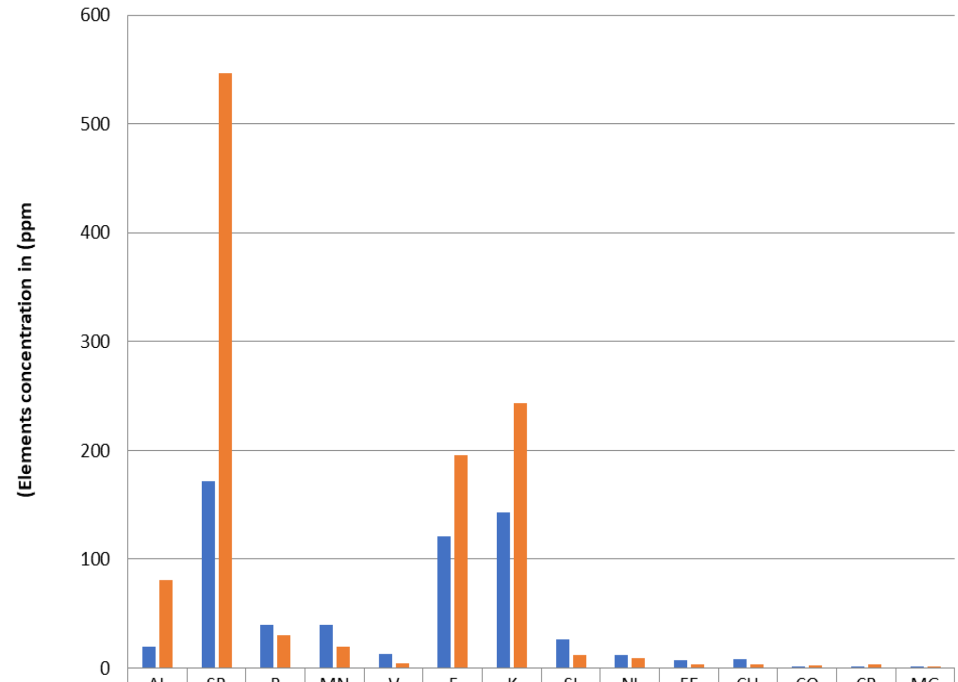


Figure 5.2: Comparisons of trace element in enamel of sound and carious Primary teeth.

Figure (5.2) compares the results obtained from the analysis of trace elements in enamel of sound and carious primary teeth. While significant differences were observed in most of trace elements, no significant differences were found between sound and carious primary teeth in amount of K, Fe, Cu & Co. Carious primary teeth have significantly higher amount of Sr ($p<0.05$). Sound primary teeth have significantly higher amount of Al, B, Mn, V, F, Si, Ni & Cr ($p<0.05$).

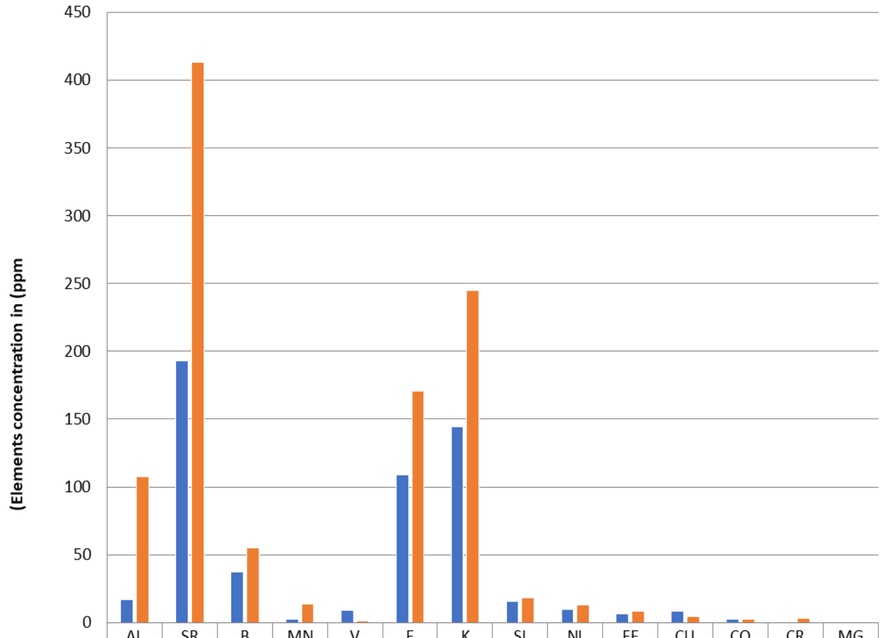


	AL	SR	B	MN	V	F	K	SI	NI	FE	CU	CO	CR	MG
Primary Sound	19.61	171.83	39.53	39.4	12.61	120.69	143.2	26.5	12.23	6.83	8.2	1.77	1.5	0.12
Permanent Sound	80.38	546.5	30.53	19.23	4.7	195.7	243.33	12.27	8.73	2.9	3.43	2.55	3.67	0.25

	P value
AL	**<0.001*****
SR	**<0.001*****
B	**0.001****
MN	**<0.001*****
V	**<0.001*****
F	**<0.001*****
K	**0.010***
SI	**0.001****
NI	**0.002***
FE	**0.001****
CU	**0.001****
CO	**0.276**
CR	**0.005****
MG	**0.003**

Figure 5.3: Comparisons of trace element in enamel of sound primary and permanent teeth.

Figure (5.3) compares the results obtained from the analysis of trace elements in enamel of sound primary and permanent teeth. While significant differences were observed in most of trace elements, no significant differences were found between sound primary and permanent teeth in concentration of Co. Primary sound teeth have significantly higher amount of B, Mn, V, Si, Ni, Fe & Cu, ($p < 0.05$). Sound permanent teeth have higher amount of Al, Sr, F, K, Cr & Mg ($p < 0.05$).



	AL	SR	B	MN	V	F	K	SI	NI	FE	CU	CO	CR	MG
Primary carious	16.7	192.7	37.6	2.77	9.03	108.91	144.37	15.73	9.67	6.33	8.37	2.31	0.67	
Permanent carious	107.5	413.27	55.03	13.6	1.38	170.54	244.67	18.37	13.1	8.23	4.5	2.7	3.17	0.17

	P value
AL	<0.001***
SR	<0.001***
B	<0.001***
MN	<0.001***
V	<0.001***
F	<0.001***
K	0.001**
SI	0.119
NI	0.011**
FE	0.011**
CU	0.022*
CO	0.491
CR	0.001**
MG	(NA)

Figure 5.4: Comparisons of trace element in enamel of carious primary and permanent teeth.

Figure (5.4) compares the results obtained from the analysis of trace elements in enamel of carious primary and permanent teeth. While significant differences were observed in most of trace elements, no significant differences were found between carious primary and permanent teeth in amount of Co & Si. Carious primary teeth have significantly higher amount of V & Cu ($p < 0.05$). Carious permanent teeth have higher amount of Al, Sr, B, Mn, F, K, Ni, Fe & Cr ($p < 0.05$).

6. Discussion

6.1. Overview

The term "trace element" is used for element either existing in nature in a small amount or that is required rather in a minute quantity[1]. In the past the trace elements were detected in small but with imprecise concentrations in the living body, but recently, with the introduction and development of the analytical technologies, such as the development of atomic absorption spectrometry, have made it possible to measure these elements precisely and to determine their functions and the characteristics of their deficiency and excess states.[156]

Although, trace elements are micronutrient chemicals but they play a vital role in maintaining the integrity of various physiological and metabolic processes occurring within living tissues and the symptoms of their under nutrition as well over nutrition may be apparent as a combination of various clinical manifestations and can result in defects of the dental hard tissues as well as oral mucosa.[5]

Dental caries is one of the most prevalent chronic diseases of people worldwide; and it is continues to be a significant dental disease and a major public health concern challenge in children and adults in developing countries.[50] Tooth structure and morphology are considered to be an important factors that may be attribute the initiation and progress of dental caries and it is believed that the presence of trace elements within the structural make-up of the enamel hydroxyapatite crystals may possibly influence the physical character of the crystals hence, rendering them more or less resistant to enamel dissolution during the initiation of the caries process.[8,41,48]

However, there are indications that trace elements play an important role in either increasing or decreasing the caries resistance of tooth structure, since various epidemiological studies have shown the role of fluorides for example, in the reduction of dental caries by altering the solubility of dental tissue and transforming the size and shape of the crystallites.[11,12,41]

Hence, the aim of this in-vitro experimental study was to estimate and compare some of trace element concentrations in sound and carious enamel of primary and permanent teeth and highlight the possible correlation between these elements and the prevalence of dental caries in children and adolescents in Benghazi city population. This study was performed on a sample composed of 48 sound and carious permanent and primary teeth which were collected from patients attended to different private and governmental dental clinics in Benghazi city. The samples were divided into four groups (A, B, C, and D) with 12 samples (n=12) included in each group according to the type of the teeth whether permanent or primary and the conditions of the teeth either sound or caries.

The concentrations of the 18 trace elements, F Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se ,Pb, Mo, and V were estimated in the different enamel samples. Three readings values were recorded, the concentrations of these elements were expressed in parts per million (ppm).
The mean, standard deviation (SD) were calculated. The values obtained were tabulated and the differences between the means in the four groups were compared using the T- test.

6.2. Comparison between trace elements in sound and carious permanent teeth:

The results indicated that the most common trace elements found in both sound and carious permanent teeth was Sr , followed by K, F and Al. On the other hand, the least observed trace elements were Mg, Cr, Co & Cu. Other elements such as Pb, Se, Zn, and Mo were not detected in either sound or carious permanent teeth as shown in Figure (5.1).

According to Table (5.1) the concentrations of Al, B, Ni, Si and Fe were significantly ($P<0.05$) higher in carious enamel of permanent teeth than the concentrations of Al, B, Ni, Si and Fe in sound enamel of permanent teeth, while the concentrations of Sr, Mn, V, and F were significantly ($P<0.05$) higher in sound enamel of permanent teeth than the concentrations of Sr, Mn, ,V and F in carious enamel of permanent teeth. So these elements may have a negative correlation with dental caries in permanent teeth.

These results agreed with previously reported findings [(15,40,41,61,100,107,164)]. Navia, 1972 listed F as a potent cariostatic element where Sr and V as low cariostatic elements in his classification of microelements and their relation to dental caries.[(40)] Shashikiran *et al.*, 2007 found that the concentrations of F, Sr and Fe were higher in sound enamel of permanent teeth than in carious enamel of permanent teeth. These findings agreed with our results regarding to F and Sr.[(41)] Curzon and Losee 1977, 1978 provided a similar results, showing a strong relationship between Sr in whole enamel and lower caries incidence in the enamel.[(84,15)]

Furthermore, Vrbic and Stupar, 1980 were able to demonstrate a negative correlation between Sr in enamel and caries incidence [(17)], which was supported by the later study of Curzon, 1985.[(85)] While, Yem *et al.*, 1991 found a probable positive association between Fe and dental caries in sample of permanent teeth.[(100)]

Srinivasarao, *et al.*, 2010 observed that concentration of Fe in caries portion of the teeth was higher than in normal enamel, dentin and cementum. They referred the accumulation of Fe on caries region to the types of food that are rich with this element more than sound enamel surface.[164]

Curzon and Losee, 1978 drew the attention that Al and B were positively related to enamel caries, as their concentrations were found evidently higher in carious enamel than in sound enamel.[15]

These findings also reported by Amin *et al.*, 2016 where they found that the concentrations of Al and Mn, were significantly higher in decayed than in normal enamel, and suggested a positive association of these three elements with caries but our findings showed that the concentration of Mn was higher in sound than in caries permanent teeth, which disagreed with the finding obtained by Amin et al., 2016.[107] On the other hand, Navia 1972 considered manganese Mn as an element with doubtful effect on caries, whereas B is mildly cariostatic element.[40]

Riyat and Sharma, 2009 found that B was significantly higher in carious teeth than non-carious teeth and considered B as one of the element that could have possible role in predisposing or causing dental caries these finding coincided with our results.[61]

Moreover, there was negative correlation between B and F levels In Lius' study in 1975 [165], B has been shown to reduce the cariostatic effect of F. It has been suggested that boron may inhibit the fluoride absorption from the gastrointestinal system. This may explained the presence of low concentration of F in carious permanent teeth by presence of high amount of B in the enamel of the same teeth group which made teeth more susceptible to the dental caries.

So the higher concentrations of these elements Al, B, Ni, Fe and Si could be one of the reasons for occurrence or predisposing dental caries in permanent teeth while, the higher concentrations of Sr, Mn ,V and F elements may have a negative correlation with dental caries in permanent teeth.

6.3. Comparison between trace elements in sound and carious primary teeth:

On-going through the results presented in Table (5.2), the results showed that the carious primary teeth have significantly higher amount of Sr ($p<0.05$) than in sound primary teeth, and the sound primary teeth have significantly higher amount of Al, B, Mn, V, F, Si, Ni & Cr ($p<0.05$) than in carious primary teeth .

These results were in concurrence with the finding of a previous report by Shashikiran *et al.,* 2007 who, illustrated that the concentration of Sr was significantly ($P <0.05$) higher in carious enamel of primary teeth than in sound enamel of primary teeth and the concentration of Si was significantly higher in sound enamel of primary teeth than in carious enamel of primary teeth.[(41)] Tanaka, *et al.,* 2004 considered Al as one of the element which may possess cariostatic properties since their findings indicated that the primary teeth containing high level of Al had less caries than teeth with lower Al concentration.[(106)]

Curzon and Crocker, 1978 found that Al may be positively associated with DMFT when interacting with Sr.[(14)] This relation was confirmed by Vrbic and Stupar, 1987, where they found a correlation to dental caries for Sr and Al.[(17)] The results of our study recorded a relation between Al and Sr concentrations in primary and permanent teeth and dental caries.

It has been suggested that fluoride could prevent caries in permanent and primary teeth.[(41,107)] The results of this study support this suggestion. This is because the fluoride level was found to be higher in sound teeth than the carious teeth in both primary and permanent groups.

Kuru *et al.,* 2020 results showed that both sound primary and permanent teeth contained more B than carious teeth. These results were agreed with our finding regarding primary teeth.[166]

The concentration of V was significantly higher in both sound primary and permanent teeth. These interesting findings were supported by the findings of Tank and Storvick, 1960 who showed a reduction in caries prevalence in both deciduous and permanent dentitions with increasing concentrations of V in drinking waters.[18] Similarly, Buttner, 1963 suggested that the cariostatic action of V seems to be associated with its uptake into enamel during tooth development; post eruptive administration has been said to enhance caries.[93]

Yem *et al.,* 1991 showed that the distribution of Ni in normal teeth was significantly higher than that in carious teeth which concluded that Ni had inverse association to dental caries.[100] This result coincided with our findings regarding Ni which found to be in high concentration in sound primary teeth. The results of Niedzielska *et al.,* 1990 concluded that the carious primary teeth contained less Mn and Ni as compared to primary teeth without caries. These finding was in agreement with our results.[167]

Al-Jorrani and El-Sammarai, 2013 reported that Cr ions played a role in improving mineralization and crystallity of teeth. This report may explain our finding of the presence of Cr in sound primary teeth with a higher concentration than in carious primary teeth.[138]

So, based on these previous reports and to our results Sr could be considered as a caries promoting element in primary teeth, whereas Al, B, Mn, V, F, Si, Ni & Cr could be cariostatic elements when they present in high concentration in primary teeth.

6.4. Comparisons of trace elements in sound primary and permanent teeth:

In most respects the mineral composition of primary and permanent teeth appear to be closely similar however, several statistically significant differences were found which evidently reflect changes in dietary habits during the development of the two types of teeth.

According to results shown in Table (5.3) the sound primary teeth have significantly higher amount of B, Mn, V, Ni, Si, Fe & Cu ($p < 0.05$) than in sound permanent teeth. These finding are in agreement with.[(8,17,41,46,49)]

Bhattacherjee and Sarkar, 1999 , Shashikiran *et al*., 2007 found that Cu, Si were significantly ($P<0.05$) higher in sound enamel of primary teeth than the concentrations of Cu and Si in sound enamel of permanent teeth.[(8,41)]

Also, Vribic and stupar, 1987 recorded the presence of a higher concentration of Cu and V in primary than in permanent teeth and higher concentration of Al and Sr in permanent than in primary teeth a result which was in coincide with our finding.[(17)]

Lakomaa and Rytomaa, 1976 and Amr and Helal, 2010 reported that enamel of primary teeth contained more Mn compared with the permanent teeth. These results is also concordat with our research findings.[(46,49)]

While sound permanent teeth had higher amount of Al, Sr, F, K, Cr & Mg ($p<0.05$) than in sound primary teeth as shown in Table (5.3). These results was in coincide with.[(41,49,167,168)]

Shashikiran *et al.,* 2007 showed that the concentrations of F, Sr, and K were significantly ($P < 0.05$) higher in sound enamel of permanent teeth than the concentrations of F, Sr, Cr and K in sound enamel of primary teeth.[(41)]

Amr and Helal, 2010 concluded that the permanent teeth contained significantly greater concentrations of Mg, Al and Sr than the primary teeth.(49) Moreover, Cutress, 1972 , Nixon and Helsby, 1976 also found that Sr was comparatively higher in enamel of permanent than in primary teeth.(168,169)

6.5. Comparisons of trace elements in primary and permanent carious teeth:

According to Table (5.4) a significant differences were observed in most of trace elements while no significant differences were found between caries primary and permanent teeth in amount of Si and Co. Carious primary teeth have significantly higher amount of V & Cu ($p < 0.05$) than in carious permanent teeth and the carious permanent teeth have higher amount of Al, Sr, B, Mn, F, K, Ni, Fe & Cr. ($p < 0.05$) than in carious primary teeth as shown in Table (5.4). These finding agreed with Shashikiran *et al.,* 2007 who, found the concentrations of F, Sr and K were significantly higher in carious enamel of permanent teeth than in carious enamel of primary teeth and significantly higher concentrations of Cu in carious enamel of primary teeth than in carious enamel of permanent teeth, but disagreed with them in presence of a significant higher concentrations of Al in carious enamel of primary teeth than in carious enamel of permanent teeth.(41)

Riyat and Sharma, 2009 found that Sr was the only element found to be present in significantly lower amount in carious teeth, thus strongly suggesting that its deficiency may predispose form caries.(61) Hence, the carious primary teeth contained significantly lower amount of Sr than carious permanent teeth, this may explain the more susceptibility of primary teeth than the permanent teeth to dental caries.

6.6. Limitations

The limitation of the present study contributed to the relatively small sample size which may limit the number of detected trace elements in enamel samples to find correlations between the concentration of trace elements and the prevalence of dental caries. Furthermore, using more advanced analytical techniques were considered to be high and not available in Libya or in nearby countries.

7.Conclusion and Recommendations.

7.1. Conclusion.

The variations in the sample population, tooth type, geographical source of the teeth, sample preparation and analytical methodology made a direct comparison of results from this work with previous studies very complex.

Within the limitation of this study it could be concluded that:

- Trace elements play important role in promoting or inhibiting dental caries.
- Fluoride concentration was higher in both sound primary and permanent teeth.
- Both V and Mn may have a cariostatic effect since they were higher in both sound primary and permanent teeth.
- Sound primary teeth had high amount of Al and low amount of Sr , while sound permanent teeth had high amount of Sr and low amount of Al.
- The concentrations of Si was significantly higher in sound enamel of primary teeth than in carious enamel of primary teeth and its concentration was higher in carious permanent teeth than sound permanent teeth. So, this could formulate the higher concentration of Si could be one of the reasons for occurrence of caries in permanent teeth and cariostatic element in primary teeth.
- Al could be a cariostatic element as it was present in high concentration in primary teeth but caries promoting element in permanent teeth.
- B could have a cariostatic effect on primary teeth.
- The Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and fluoride Ion selective electrode techniques were perfectly suited for analysis of the trace elemental in dental tissue hence, they profound the relatively same results obtained by other techniques used in previous researches.

7.2. Recommendations

1. It is recommended more encouraging regular application of fluoride due to its ability to increase tooth resistance against dental caries.
2. Further studies should be performed to compare trace element concentrations in sound and caries enamel of primary and permanent teeth based on a large sample or scale on different geographic areas.
3. Expand the scope of the research to include more trace elements and their correlations between their concentrations and the incidence of dental caries.
4. Create a mixture of these elements at an optimal levels and ratios to find their roles and combined effects on promoting or inhibition of dental caries.
5. Use more advanced means of analytic technique for trace elements detection to confirm and compare between different results.

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Consent Form **Appendix 1**

استمارة موافقة على المشاركة في دراسة بحثية

أنا الباحثة أميمة عبدالحميد بن سليم , طالبة دراسات عليا بكلية طب اسنان جامعة بنغازي قسم طب أسنان أطفال, أقوم ببحث عن **بعض العناصر النزرة في مينا الأسنان اللبنية والدائمة المتسوسة والسليمة لدى الأطفال في مدينة بنغازي**

قد تم اختياركم للمشاركة في هذا البحث مع عدد اخر من المشاركين في مدينة بنغازي, وحيث أن أهداف هذا البحث تقييم معدلات هذه العناصر في مينا الأسنان لدى الأطفال.

ونتوقع بمشاركتك والأخرين أن نحصل علي نتائج تفيد الخدمات الصحية ومعرفه أسباب المشاكل الصحية للفم, وكذلك تقديم توصيات من شأنها المساهمة في رفع مستوي الخدمات المقدمة للناس.

ومن خلال هذه الدراسة سأقوم بالعمل على المحتوي الغير عضوي للأسنان وتحليلها لمعرفة المكونات الأساسية وتركيزها داخل الأسنان الذي سيساهم في تطوير الخطط الوقائية لعلاج مشاكل الأسنان, مع العلم أن الإجراء الذي سأقوم به يخلو من أي مخاطر علي المشارك.

ونحن إذ نأمل منكم المشاركة معنا في هذا البحث , نؤكد لك على سرية المعلومات والوثائق الخاصة بك, وأنه لن يطلع عليها إلا الباحث المعني.

أسم المشارك :.......................... **التوقيع** :............................

شكرا لحسن تعاونكم.

Appendix 2

STUDY OF SOME TRACE ELEMENTS IN SOUND AND CARIOUS ENAMEL OF PRIMARY AND PERMANENT TEETH OF CHILDREN IN BENGHAZI CITY

دراسة بعض العناصر في مينا الأسنان اللبنية والدائمة المتسوسة والسليمة لدى الأطفال في مدينة بنغازي

By

Omima Abdulhamid Omran

(BDS,2000)

Protocol

Submitted in Partial Fulfilment of the Requirements For

The Degree of Master of Science in Paediatric Dentistry

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1. Introduction

Human teeth are a complex system of specialized tissues like enamel, dentin, cementum, and pulp. Basically Each tooth has two parts: the crown and the root. The dental enamel is the outer layer which covers the anatomical crown of human teeth and protect the underlying tooth structure [(1)].

Dental enamel is the hardest mineralized tissue in human body .where it is contains about 95% inorganic mineral and 3%water and 2% organic material [(2)].

The inorganic component of human enamel is consisted mainly of a crystalline form hydroxyapatite (HAP) [(3)]. The HAP crystals contain various trace amounts of inorganic elements and the elements which are present in less than 0.01% (i.e., in micrograms per gram) are the trace elements. These trace elements in the human dental enamel are derived from the environment during mineralization and during and after maturation of tooth [(4,5)]. It has been founded that the physical and chemical properties of enamel is changed by presence of these trace elements [(6)].

Since dental enamel has special property of sequentially calcifying growth layers that can provide an archival record of environmental changes such as changes in elemental nutrition even past pollution events can be determined on that tissue [(7)].

So, the retrospective look on this archive could provide a historic record of these trace elements and useful information could be obtained about the relation between caries formation process and presences of these trace elements ,so analysis of chemical element content of teeth can expands the knowledge of etiology of dental diseases and it can be used for diagnostic, therapeutic and preventive purposes [(8)].

2. **Review Of Literature**

Several studies were conducted to analyze the composition of human teeth and the researchers found that dental tissues contain a large number of minor inorganic constituents and trace elements in a varying quantity such as fluoride (F), strontium (Sr), vanadium (V), molybdenum (Mo), zinc (Zn), copper (Cu), manganese (Mn) and lead (Pb) in addition to the major components [(9,10)].

The investigators trying to find link between trace elements and dental caries by study human teeth as several epidemiological studies have proved that a trace element such as fluoride can alter the solubility of dental tissue and transforming the size and shape of the crystals which makes enamel more resistant to the dental caries [(4,11-14)].

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 Se,Mg,Cd,Pb,Pt,Si; and uncertain Br,Be,Co,Mn,Sn,Zn,and I [(15)].

While others proposed another classification where they categorized them as Cariostatic elements. Fluoride (F), Phosphorus (P).

Mildly cariostatic. Mo, V, Cu, Sr, B, Li, Au

Doubtful. Be, Co, Mn, Sn, Zn, Br, I.

Caries inert. Ba, Al, Ni, Fe, Pd, Ti.

Caries promoting. Se, Mg, Cd, Pt, Pb, Si [(16)].

Others found out that the presences of F, Al, Fe, Se and Sr were associated with the low risk of tooth caries, while Mn, Cu and Cd had been associated with a high risk [(12)]. On the other hand, a different study showed that the correlation between the amounts of aluminium, barium, copper, and zirconium in enamel caries was not significant [13] while a negative correlation was observed between strontium and enamel caries in extra study [17]. An additional study concluded that some other trace elements besides fluoride play an important role in the caries process [18]. Other interesting study showed a reduction in caries prevalence in both deciduous and permanent dentitions with increasing concentrations of V in drinking water [19].

Since, the dental caries is the most common chronic childhood disease in the United States The prevalence of dental caries exceeds 50% in 5- to 9-year-old U.S. children and increases to 78% in those 17 years of age, making this disease more common than asthma and hay fever [20].

So the aim and objective of this vitro study is to estimate and compare the trace element concentrations in sound and carious enamel of primary and permanent teeth and finding the relation between these elements and prevalence of dental caries in children and adolescents of Benghazi.

3. Aim of the study:

This study is designed to:

1. Estimate and compare the trace element concentrations including the fluoride in sound and carious enamel of primary and permanent teeth in samples of children from Benghazi, and finding their relation to dental caries.
2. Assess the need of fluoride use in Libyan children.
3. Provide a basic line data and encourage for any future study.
4. Provide information for the health authority for planning any preventive dental programs.

4. Materials and Methods

4.1 Materials

This in vitro study Forty eight extracted sound and carious primary and permanent teeth going to be collected from children and adolescents of Benghazi city; the selection criteria will be as follows:

The selection criteria were as follow:

- **Inclusion criteria**

a) Sound primary incisors that had been extracted for reasons of pre- shedding mobility or over-retention in children with an age-group of 7-12 years.

b) Carious primary incisors that had been extracted for reasons of carious involvment, pre-shedding mobility or over-retention in children with an age-group of 7-12 years.

c) Sound premolars that had been extracted for orthodontic reasons in adolescents with an age-group of 14-20 years.

d) Carious premolars that had been extracted for carious destruction in adolescents with an age-group of 14-20 years.

e) The teeth were taken from patients borne and grow up in Benghazi city.

- **Exclusion criteria**

a) Extracted teeth with developmental anomalies and fluorosis.

b) Teeth extracted from patients suffering from systemic diseases.

C) The extracted teeth had no previous restorations.

4.2 Method

4.2.1 Study Design:

An in-vitro experimental study will be performed to assess the trace elements in 48 samples of an enamel powder which were collected from sound and carious permanent and deciduous teeth by using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Analysis and Fluoride ion selective electrode.

4.2.2. Consent form

information sheet that explained the purpose of the project have to be given to the participants and signed written informed consents will be obtained from all the subjects selected for the study.

4.2.3 Sample collection and preparation:

Washing the extracted teeth thoroughly under tap water to remove saliva, blood, and tissue debris then removing the soft caries from the carious teeth by using a sharp spoon excavator.

Dividing the teeth into 4 groups

Group A: 12 sound primary incisors.

Group B: 12 carious primary incisors.

Group C: 12 sound permanent premolars.

Group D: 12 carious permanent premolars.

Enamel samples will be prepared by mechanical grinding [(15)], leaving the dentinal portion intact by using a cylindrical alpine tooth grinding stone fitted to a straight hand piece the whole grinding procedure going to be undertaken inside a transparent plastic bag to prevent the resulting enamel dust from becoming air-borne and lost,. The speed of grinding will be at a about 10,000 rpm [4,21].

The pooled enamel dust going to be collected on a plastic sheet at first and then transferred to clean plastic containers labelled A, B, C, and D

- Preparation of solution for TEs analysis by ICP-OES:

One gram of enamel dust from enamel sample A will be taken in a volumetric flask and was wet-ashed in 2 ml of nitric acid to produce a clear solution[22].

Further Diluting the solution by using double-distilled deionized water to make the volume 100 ml. so, the final solution contains nitric acid at a concentration of 1M (Molar solution), labelling the prepared solution as solution A.

- Enamel samples B, C, and D will similarly processed to make solutions B, C, and D, respectively.

- Preparation of solution for F analysis by Fluorid SE meter:

One gram of enamel powder from sample A should wet-ashed in concentrated in concentrated HCl then, a 900 μL sodium acetate solution with concentration 15 % will added to each 100 μL of dissolved enamel samples to increase the pH of the solution, Then, 900 μL of this solution will be taken and 100 μL of TISAB-II buffer solution should added to adjust the total ionic strength, and release the F ions and hence increases the accuracy of the reading [23].

The same procedures will be applied on samples B,C and D to make final solutions B, C, and D, respectively.

The concentrations of the, Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se, Pb, Mo, and V will be estimated in all four enamel samples by ICP-OES while F will be estimated by using Fluorid SE meter.

5. References

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Lap Results
Appendix 3

Ministry of Scientific Research
Central Metallurgical R & D Institute
Technical Services Department

CMRDI

وزارة البحث العلمي
مركز بحوث وتطوير الفلزات
إدارة الخدمات الفنية

تقرير اختبار التحليل الكيميائي للعناصر بالحث المزدوج البلازمي (ICP)

رقم التقرير	7	تاريخ إصداره	2017/6/ 1
اسم العميل	د.سالم السيد احمد فتح لله		
عدد العينات	4	شكل العينات	بودرة
بيانات العينات	بودرة	الرقم الكودي للعينات	17/ 1294:1297
تاريخ استلام العينات	2017/4/3	تاريخ إجراء الإختبار	2017/4/3
الظروف البيئية	درجة الحرارة : 22.8 درجة مئوية	الرطوبة :31	
اسم الجهاز المستخدم	ICP-OES Optima 2000 DV Perkin Elmer		
رقم الملف	449		

نتائج الإختبـــــــار

(التحليل الكيميائي ppm)

Sample ID	Sample name	Al			Sr			B			Mn			V		
17/1294	A	18.90	19.88	20.05	171.0	171.5	173.0	38.4	39.7	40.5	38.54	39.76	39.91	12.72	12.4	12.72
17/1295	B	16.1	16.8	17.2	187	194	197	37.1	37.8	37.9	3.3	3.6	1.4	8.7	9.0	9.4
17/1296	C	79.7	80.38	81.05	543.7	546.5	549.3	29.3	30.8	31.5	18.7	19.3	19.7	4.1	4.8	5.2
17/1297	D	105.1	107.7	109.7	406.1	415.4	418.3	53.9	55.4	55.8	12.7	13.8	14.3	1.02	1.23	1.83

القائم بالعمل	المسئول الفني	مدير إدارة الخدمات الفنية
ك. محمود عبد الجواد	ك. هدى طه عبدالرحمن	م. ناصر جمعه

رقم الصفحة	1
عدد الصفحات	4

نتائج الإختبـــــــار

Sample ID	Sample name	Co			Cr			Mo	Mg		
17/1294	A	1.3	1.6	2.7	1.2	1.4	1.9	Not detected	0.11	0.12	0.12
17/1295	B	1.6	2.1	2.4	0.4	0.7	0.9	Not detected	Not detected		
17/1296	C	1.9	2,8	3.5	2.7	3.2	3.6	Not detected	0.21	25.	0.28
17/1297	D	2	2,4	3.1	3.2	3.5	4.3	Not detected	0.13	0.18	0.2

القائم بالعمل	المسئول الفني	مدير إدارة الخدمات الفنية
ك. محمود عبد الجواد	ك. هدى طه عبدالرحمن	م. ناصر جمعة

رقم الصفحة	2
عدد الصفحات	4

نتائج الاختبـــــــــار

Sample ID	Sample name	Pb	Se	Zn
17/1294	A	Not detected	detected Not	Not detected
17/1295	B	Not detected	Not detected	Not detected
17/1296	C	Not detected	Not detected	Not detected
17/1297	D	Not detected	Not detected	Not detected

القائم بالعمل	المسئول الفني	مدير إدارة الخدمات الفنية
ك. محمود عبد الجواد	ك. هدى طه عبدالرحمن	م. ناصر جمعة

رقم الصفحة	3
عدد الصفحات	4

نتائج الإختبـــــــار

Sample ID	Sample name	K			Si			Ni			Fe			Cu		
17/1294	A	120.4	139.2	170	23.7	26.5	29.3	11.6	12.2	12.9	6.2	6.8	7.5	7.5	8.2	8.9
17/1295	B	138.8	145.8	148.5	14.1	15.8	17.3	8.7	9.9	10.4	5.7	6.4	6.9	6.7	8.3	10.1
17/1296	C	217	239	274	10.6	12.8	13.4	8.2	8.7	9.3	2.5	2.8	3.4	2.7	3.4	4.2
17/1297	D	223	246	265	16.8	18.2	20.1	12	13.4	13.9	7.9	8.1	8.7	4.0	4.2	5.3

تم إذابة العينات بإستخدام حمض النتريك وحمض الهيدروكلوريك.

- نتائج الإختبارات تمثل العينة المقدمة للمركز فقط ولا تمثل الإنتاج او أي كميات اخري طرف جهة تقديم العينة.
- التقارير التي يصدرها المركز سرية وتخص الجهة الطالبة وحدها ولا يجوز إعطاء صورة منها لأي جهة اخري.
- تنقضي مسؤولية المركز عن تسليم باقي العينات للعميل خلال 30 يوما من صدور التقرير أو إستنفاذ العينة في الإختبارات.

قائم بالعمل	المسئول الفني	مدير إدارة الخدمات الفنية
. محمود عبد الجواد	ك. هدى طه عبدالرحمن	م. ناصر جمعة

رقم الصفحة	4
عدد الصفحات	4

intertek

Total Quality Assured.

Intertek – Analysis Report

Job number: 17-01616 **Client:** Dr. Salem Elsayed

Job description: Enamel **Client Reference:** N/A

Grade: Chemicals **Date Received:** 12/11/2017

Date completed: 12/11/2017

Test	(A)			(B)			(C)			(D)		
Fluoride (ppm)	118.8	120.1	123.15	105.8	108.9	112	191.7	196	199.2	164.8	171.2	175.6
Sample Number	*17-01616-01*			*17-01616-02*			*17-01616-03*			*17-01616-04*		

Notes : **Instrument : HANNA / HI 98402 ISB Meter Fluoride**

Test Method : SM4500 F – C

ITS CBEL Manager

Hatem Hasseiny

intertek

Total Quality Assured

Alexandria, Egypt

infoCAA-Egypt@ intertek.com

دراسة بعض العناصر في مينا الأسنان اللبنية والدائمة المتسوسة والسليمة لدى الأطفال في مدينة بنغازي

قدمت من قبل

أميمة عبد الحميد عمران بن سليم

تحت إشراف

أ.د. نجاة حسن بويطينة

الملخص

الخلفية والهدف : مينا الأسنان هي الجزء الأكثر صلابة في جسم الإنسان والذي يغطي التاج التشريحي للسن وتحتوي على عدد كبير من المكونات غير العضوية والمعادن بكميات متفاوتة ، ونظرًا للأهمية الأساسية لهذه العناصر في عدد من العمليات الكيميائية الحيوية والفسيولوجية لدى الإنسان ، فمن المعقول افتراض أنها قد تؤثر على الآليات المسؤولة عن زيادة التسوس أو تثبيطه والذي يعتبر بدوره أكثر أمراض الفم شيوعًا والسبب الرئيسي وراء فقدان الأسنان والثالث بين الأمراض غير المعدية التي تهدد صحة الإنسان.

حيث وجد أن قابلية ذوبان المينا لهجمات الحمض الناتج من مخلفات البكتيريا المتراكمة على سطح السن تعتمد على وجود نسبة أعلى أو أقل كمية من هذه العناصر في مينا الأسنان والتي تعمل على الأرجح من خلال تغيير مقاومة الأسنان نفسها أو عن طريق تعديل البيئة المحيطة بالسن أو التركيبية الكيميائية والفيزيائية للأسنان.

حتى اليوم لم يتم بعد تحديد أهمية هذه العناصر والتي تتواجد بكميات دقيقة في جسم الانسان , في الوقاية من تسوس الأسنان والحد منه بشكل كامل على الرغم من إجراء دراسات مختلفة للعثور على الصلة بين هذه العناصر وتسوس في أسنان الإنسان لهذا اجريت هذه الدراسة التى كان الهدف منها تقدير بعض العناصر في مينا الأسنان السليمة والمتسوسة للأسنان اللبنية والدائمة للأطفال في بنغازي وتقييم الارتباط بين وجود التسوس وهذه العناصر.

المواد والطرق: تم تقدير تركيزات العناصر الثمانية عشر ، F، Sr، K، Al، Si، Ni، B، Fe، Cu، Mn، Co، Cr، Zn، Mg، Se، Pb، Mo, و V

بواسطة استخدام جهاز (ICP–AES)، وجهاز (FSE meter) في عينة مكونة من 48 سن سليمة ومتسوسة دائمة ولبنية تم جمعها من المرضى المترددين على عيادات الأسنان الخاصة والحكومية المختلفة في مدينة بنغازي , حيث تم طحن المينا وتقسيمها إلى أربع مجموعات (أ ، ب ، ج ، د) كل مجموعة تحتوي على مسحوق ل 12 سن (ن = 12) مجموعة حسب نوع الأسنان سواء كانت دائمة أو لبنية وظروف الأسنان سواء سليمة أو بها تسوس.

النتائج: أوضحت الدراسة أن العناصر الأكثر شيوعًا التي تم اكتشافها في كل من الأسنان الدائمة السليمة والميسرة في هذه الدراسة كانت Sr ، يليه K و F و Al بينما أقل العناصر الملحوظة كانت Cr, Mg, Coو Cu ظهرت النتائج أيضًا أن العناصر الأكثر شيوعًا الموجودة في كل من الأسنان اللبنية السليمة والمتسوسة كانت Sr ، يليها K و F و B بينما كانت العناصر الأقل ملاحظة هي Mg و Cr و Co و Fe بمقارنة النتائج ، تم التوصل إلى أن الأسنان اللبنية السليمة تحتوي على كمية أعلى بكثير من Mn ، V، B، Si، Fe، Cu و Ni.

اما الأسنان الدائمة السليمة فتحتوي على كمية أعلى من Al ، Sr، Cr، F، K، Mg بينما الأسنان اللبنية المتسوسة أظهرت كمية أعلى من Cu و V

احتوت الأسنان الدائمة المتسوسة على كمية أعلى بكثير من Ni، Fe، Al ، Sr، B، Mn، K، F و Cr.

استنتاج: نتائج هذه الدراسة عززت ما توصلت إليه البحوث السابقة عن أهمية الفلورايد كعامل وقائي ضد تسوس الأسنان. علاوة على ذلك ،و بجانب F ، قد يكون لكل من العناصر V و Mn تأثير مضاد للتسوس. يمكن أن يكون التركيز العالي لـ Si أحد أسباب حدوث تسوس الأسنان الدائمة وكعنصر يمنع تسوس الأسنان اللبنية بينما يمكن أن يكون Al عنصر مثبط في عملية التسوس نظرا" لوجوده بتركيز عالٍ في الأسنان اللبنية السليمة ولكنه يزيد من التسوس في الأسنان الدائمة . كما يمكن أن يكون لمعدن ال B تأثير مضاد للتسوس في مينا الأسنان اللبنية .

الكلمات المفتاحية : المينا ، العناصر ، تسوس الأسنان.

دراسة بعض العناصر النزرة في مينا الأسنان اللبنية والدائمة المتسوسة والسليمة لدى الأطفال في مدينة بنغازي

قدمت من قبل

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تحت إشراف

أ.د. نجاة حسن بوبطينة

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير

في طب أسنان الأطفال

كلية طب وجراحة الفم الأسنان

جامعة بنغازي

2022