



Quantitative Analysis of Trace Elements in Sound and Carious Enamel of Primary and Permanent Dentitions

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Authors' contributions

This work was carried out in collaboration among the three authors. Author WA designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author FA managed the literature searches, participated in the statistical analysis, and had inputs in the manuscript. Author MA supervised the running of the GFAAS tests on some samples and managed data collection. Author WA proofread and prepared the manuscript for publication. The three authors read and approved the final manuscript.

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ABSTRACT

Aims: Quantitative assessment of trace elements in enamel of sound and carious permanent and deciduous teeth, and evaluation of correlation between the presence of caries and trace elements

Study Design: Using powdered enamel from extracted primary and permanent human dentitions and graphite furnace atomic absorption spectrophotometry.

Place and Duration of Study: Faculty of Dentistry and clinical Dentistry section of the Jordan University Hospital, between October 2014 and May 2015.

Methodology: Forty extracted human deciduous and permanent dentitions were collected from patients attended the dental surgery and pedodontic departments of the Jordan University

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Hospital. After proper cleaning and drying, four groups of ten teeth each from which enamel samples were collected in four containers labelled as, sound permanent, carious permanent, sound primary, and carious primary enamel. In each group the corresponding teeth were mechanically ground using a different bur; the grinding was within the enamel layer and without exposing dentine. The powdered enamel samples were acid-digested and transferred to liquid solutions in order to measure their content of heavy metal ions by means of Atomic Absorption Spectrophotometer (GFAAS).

Results: Eight trace elements were detected, namely, Cu, Pb, Sr, Fe, Mn, Zn, Al, and Se and their concentrations were estimated in all four enamel samples. The concentrations of Sr, Cu, and Fe were significantly higher in sound than in carious enamel of primary and permanent teeth. The concentrations of Al, Mn, and Se were significantly higher in carious than in sound enamel of primary and permanent teeth. Zn and Pb demonstrated comparable concentrations in carious and normal enamel of primary and permanent teeth.

Conclusions: In the light of the obtained results, it could be concluded that Sr, Cu, and Fe function as cariostatic elements and their presence enhances caries resistance of primary and permanent teeth, whereas, the presence of Al, Mn, and Se is associated with caries.

Keywords: Trace elements; cariostatic; cariogenic; atomic absorption spectroscopy.

1. INTRODUCTION

The enamel of human teeth is composed mainly of inorganic minerals (96%) organized in a crystalline structure embedded in a very small organic matrix (4%) and water [1]. Inorganic mineral content of the enamel is mainly made of Calcium and Phosphate. However, trace elements are also present in the inorganic and may be in the organic portion as well. They integrate into enamel tissue either during tooth development phase, or after tooth eruption in the oral cavity [2].

Various analytical methods have been used in determining trace element content in enamel. These included atomic absorption spectrophotometry (AAS), [3–6], proton induced x-ray emission [7,8], inductively coupled plasma-mass spectrometry [9,10], X-ray fluorescence [11], and x-ray microtomography [12].

Many researchers evaluated the concentration of trace elements in teeth and studied their effect on enamel resistance to caries [13–23]. Some investigators attempted to establish a link between the presence of specific trace elements and enamel susceptibility to caries. Elements, such as, Mn, Mo, V and Sr, were reported to be cariostatic or playing a role in increasing caries resistance of enamel [3,4,15]. On the other hand, Se, Cu and Cd were shown to have positive association with caries [6,12,15,22,23], whereas, no such association was found with regard to other trace elements; for example, Ni and Ba [6].

The choice of a particular technique for analysis of trace elements depended on its sensitivity,

reproducibility as well as on its convenience and availability.

The aim of the present investigation is twofold, firstly, to detect, estimate, and compare the concentration of some trace elements in the enamel of sound and carious permanent and deciduous teeth of a random sample of the Jordanian population. Secondly, to highlight the possible correlation between the presence of enamel caries and trace elements constituent in deciduous and permanent enamel using atomic absorption spectrophotometry.

2. MATERIALS AND METHODS

Forty extracted human deciduous and permanent dentitions were collected from patients attended the dental surgery and pedodontic departments of the Jordan University Hospital. Prior to conducting the investigation, and in compliance with the policy of the Clinical Research Authority at the JUH, signed written informed consents were obtained from all the subjects selected for the study. All subjects were made aware that their extracted teeth, tissue biopsies and radiographic images were included in this investigation and could be used in other research studies. The experimental protocol was examined and approved by the hospitals' Ethics Committee (JUHEC) and was, therefore, performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki [24].

The collected teeth had no previous restorations; they were devoid of any developmental

anomalies and were taken from patients who had no history of systemic diseases.

The teeth were cleaned under running tap water to remove debris and blood. Soft caries and remaining tissues attached to the teeth were removed with a large spoon excavator and a sharp curette. Teeth were then kept for two hours in an "enzymatic solvent" (Asepti-Zyme" presoak cleaner (Ecolab Inc. MN USA)) to dissolve persistent organic matter. They were, then washed in tap water to remove the enzyme and stored under water in glass containers. The teeth were dried in open air for about 24 hours before the enamel sample collection, and were divided into four groups of ten teeth each. Group 1 contained sound permanent dentitions; group 2 contained carious permanent dentitions; group 3 contained sound primary dentitions; and group 4 contained carious primary dentitions (Fig. 1 A-D). Each tooth was then mounted in an acrylic block to facilitate the grip during enamel sample collection. A large cylindrical grit Alpine stone bur (SS White Burs, Inc. Lakewood, NJ USA) mounted on a straight laboratory hand piece was used to mechanically grind the teeth at a set speed of 10,000 rpm; to avoid heat and charring.

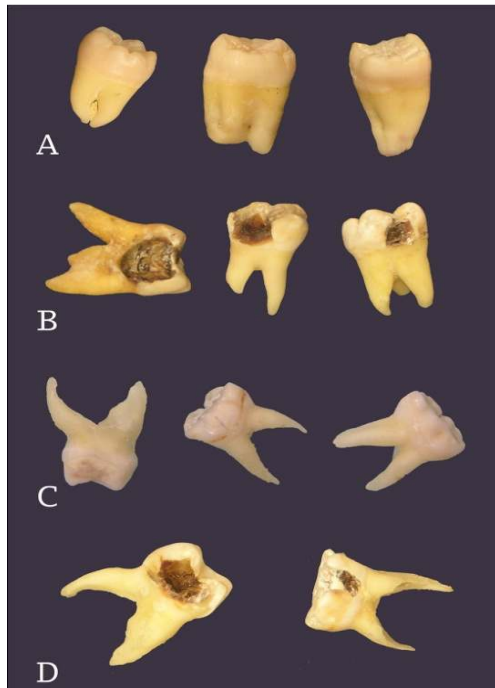


Fig. 1. Sample of extracted whole teeth before powdering their enamel tissue; showing sound permanent (A), carious permanent (B), sound primary (C), and carious primary teeth (D).

Enamel grinding was carried out inside a thick cardboard box lined and covered with a plastic sheet in order to prevent enamel dust becoming airborne. Specimens of each group were mechanically ground using a different bur and a different container, to avoid 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 box was kept in sterile "Petri dish" (Fig. 2).

2.1 Sample Digestion Procedure

The aim of the digestion process of enamel samples was transferring the enamel powder sample to a liquid solution in order to measure their content of heavy metal ions by means of Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS).

One gram of enamel powder from group 1 enamel sample was digested for two hours at $160^{\circ}\text{C}\pm 10^{\circ}\text{C}$ in 8 ml concentrated Perchloric acid and 2 ml concentrated Nitric acid with continuous stirring using mechanical stirrer until a clear solution was produced. The prepared solution was transferred to a 50 ml volumetric flask and diluted to the mark with deionized water. One ml of the sample's final solution was then transferred to 10 ml volumetric flask and further diluted.

Enamel powder samples of groups 2, 3, and 4 were similarly processed to make solutions samples of groups 2, 3, and 4, respectively.



Fig. 2. A sterile petri dish containing powdered sound primary enamel sample. Similar dishes were prepared for carious primary, sound permanent and carious permanent enamel samples.

Standard solutions for the analyzed eight elements: Cu, Pb, Sr, Fe, Mn, Zn, Al, and Se, which contained about 100 µgm/ml of the particular element, were commercially prepared by Fluka Company (Switzerland). The working standard solutions were prepared by suitable dilution of the standard solutions for each element to be analyzed.

The Graphite furnace atomic absorption spectrophotometer (GFAAS) used was the AI 1200 model from Aurora Company (Canada).

This model is directly controlled by a computer and equipped with a laser printer. The GF instrument needs Argon (99.999% purity) as a purge gas. The AI instrument is equipped with a hollow cathode lamp (HCL) turret, which can hold five lamps, and each HCL is held in the turret by an individual housing. By using the AI instrument, the atomic absorption or the atomic emission can be measured via a single beam mode or a double beam mode.

2.2 Principle of Atomic Absorption Spectrophotometry (AAS)

When a solution containing a metallic salt is aspirated into a flame (e.g., acetylene burning in air), a vapor, which contains atoms of the metal, is formed. However, a much larger number of the gaseous metal atoms will normally remain at the ground state. These ground state atoms are capable of absorbing radiant energy of their own specific resonance wavelength that, in general, is the wavelength of the radiation that the atoms would emit if excited from the ground state. Hence, if light of the resonance wavelength is passed through a flame containing the atoms of the element being detected, then part of the light will be absorbed, with the extent of absorption being proportional to the number of the ground-state atoms present in the flame. The amount of absorption of light energy by the particular element is measured through AAS.

2.3 GFAAS Operating Conditions

The measurement of heavy metals concentrations in the standard solutions and the samples was carried out using a GFAAS, which utilizes Argon as a purge gas (Table 1).

The enamel samples were analyzed for their content of trace elements according to the heating programs recommended by the manufacturers.

2.4 Measurement of Heavy Metals Concentrations in the Four Investigated Samples

The samples were injected into a pyrolytically coated graphite tube of 5.7 mm internal diameter and 19 mm length by means of XYZ auto-sampler with a small volume (10-15 µL). The graphite tube was heated in programmed steps to achieve three principle stages. First, the drying step that aimed to remove all the solvent from the sample by slow boiling the solvent away. Second, the ashing (or charring or pyrolysis) step which removed any dry or semi-dry matrix from the sample that was left over from the drying step. Third, the atomization step that converted all sample compounds into the ground state analyte atoms that absorbed part of the light, which is generated by the HCL. The sample vapor was purged from the graphite furnace by argon gas to prepare the tube for the following injection. The amount of light energy absorbed by the atomized atoms depended on the concentration of the atoms in the sample. The magnitude of the sample absorbance was recorded as a function of concentration by the read-out system, then the concentration of the unknown sample was calculated from the linear equation between absorbance and concentration.

2.5 Calibration Method

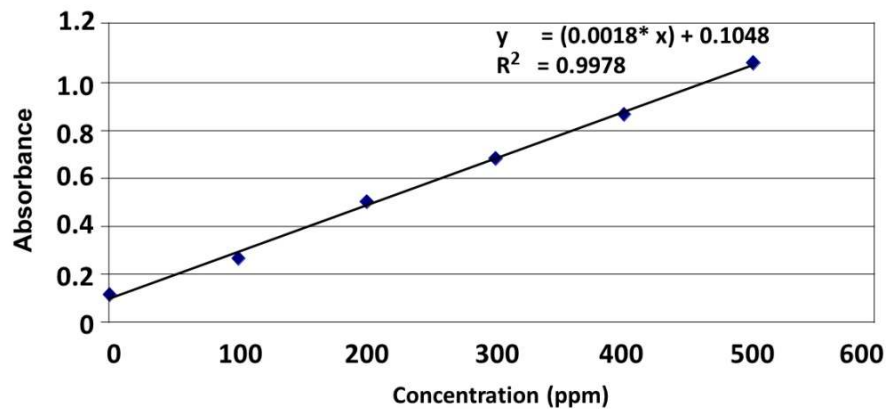
The stock standard solutions of the heavy metals, that were used, were available commercially in 1000 ppm concentrations. Series of standards were prepared from the 1000 ppm stock standards by serial dilutions in order to minimize errors. The series of standards would bracket the concentration range where the teeth samples concentrations were expected to fall. All working standard solutions that were used in the analysis were prepared from the stock standards. A plot of absorbance versus concentration was constructed for the several standards and the curve that gave the best linear equation was drawn. Then, the concentrations of tooth samples were determined from substituting the signal response of the unknown sample into the linear calibration curve then the concentration of the unknown sample was calculated, an example of a linear calibration curve is demonstrated by Fig. 3.

2.6 Statistical Treatment

The concentrations of the eight trace elements, namely, Cu, Pb, Sr, Fe, Mn, Zn, Al, and Se were

Table 1. Optimized instrumental conditions for GFAAS

Element	Current of HCL (mA)	Slit width (nm)	Wave length (nm)	Purge gas	Background correction	Injected volume (µl)	PMT voltage
Cu	6.2	0.6	228.8	Argon (Ar)	None	10	573
Fe	5.0	0.6	248.3	Ar	None	90	380
Sr	10	0.6	460.7	Ar	None	10	330
Pb	10.5	0.6	217.0	Ar	None	10	500
Mn	3.8	0.2	279.5	Ar	None	20	360
Zn	3.0	0.2	213.9	Ar	None	10	367
Se	17.7	1.2	196.0	Ar	None	15	605
Al	3.0	.2	309.3	Ar	None	10	376

Cu**Fig. 3. Calibration curve for Copper**

estimated in all four enamel samples. Five readings were recorded for each element (in the same sample synchronously), the mean, standard deviation (SD), and range were calculated. The obtained values were analyzed and differences between the means in the four groups were compared for each element using *t'* test at $p < 0.05$ level of statistical significance.

3. RESULTS AND DISCUSSION**3.1 Results**

The results of the present investigation revealed the presence of eight trace elements (Cu, Pb, Sr, Fe, Mn, Zn, Al, and Se) in enamel samples of intact and decayed deciduous and permanent teeth. The concentrations of some other trace elements, namely, Co, B and V were below the detection limit (< 0.1 ppm) of the employed AAS equipment.

The calculated concentration mean, and standard deviation of the detected element in the four enamel samples are shown in Table 2.

The variations in trace elements concentrations in the four investigated enamel samples are shown in Table 2 and Figs. 4 and 5.

The Zinc, Zn, was the highest of all the other seven detected elements in the four enamel samples. The differences in the concentration of Zn between sound and carious enamel of both primary and permanent teeth were marginal and statistically insignificant differences (Fig. 4).

Similar trend was demonstrated by Pb, which did not show significant concentration differences neither in sound nor in carious enamel of both primary and permanent teeth (Fig. 5). Each of the two elements, Zn and Pb, revealed concentration in sound permanent enamel comparable to concentration in sound primary enamel. Moreover, the concentration of each element in the carious permanent enamel was also comparable to its concentration in the carious primary enamel (Figs. 4, 5).

The other six detected elements could be categorized in two groups. The first comprised

three elements, Sr, Cu, and Fe, all of which demonstrated significantly higher concentrations in sound than in carious enamel of both primary and permanent teeth (Table 2). Strontium, Sr, showed a significant 26% higher concentration in sound permanent tooth enamel than in the carious enamel counterpart; and a significant 15% higher concentration in sound than in carious enamel of primary teeth (Table 2). The concentration of Sr in like enamel samples, i. e., sound or carious, were comparable regardless whether the teeth were primary or permanent (Fig. 4).

Copper, Cu, demonstrated a significant 18% higher concentration in sound permanent than in carious permanent enamel; and a significant 31% higher concentration in sound than in carious enamel of primary teeth. Copper showed comparable concentration in like enamel samples (sound or carious) regardless the type of teeth (Fig. 4).

Iron, Fe, exhibited a similar trend to the other elements of the group and showed a significant 28% higher concentration in sound than in carious permanent enamel; and a significant 17% higher concentration in sound than in carious primary enamel samples (Fig. 4).

The second group of elements included Al, Mn, and Se which, unlike the elements of the first group, these revealed higher concentrations in carious than in sound enamel of both primary and permanent teeth (Table 2).

Aluminum, Al, concentration in carious permanent enamel was about 5% higher than that of the sound permanent enamel. Al, showed of a significant 8% higher concentration in carious primary tooth enamel than in sound enamel counterpart. This element demonstrated higher concentrations in primary tooth enamel than in permanent tooth counterparts. These

differences were significant in both sound enamel and in carious enamel (Table 2).

Manganese, Mn, showed a significant 20% higher concentration in carious than in sound permanent enamel; and a significant 13% higher concentration in carious than in sound primary tooth enamel. Likewise, Aluminum, the concentrations of Mn were significantly higher in primary than in permanent tooth enamel, regardless the status of enamel whether sound or carious (Fig. 5).

Selenium, Se, demonstrated similar behavior to the other elements of the group and revealed a significant 38% higher concentration in carious than in sound permanent enamel; and a significant 27% higher concentration in carious than in sound primary tooth enamel. Selenium showed comparable concentrations in like enamel samples (Fig. 5).

3.2 Discussion

The initiation and progress of dental caries may be attributed to many factors, among them, standard of oral hygiene, patient's dietary habits, patient's age and sex, origin of the drinking water, tooth morphology, and many others. In addition, it is believed that the presence of trace elements as parts of the structural make up of enamel may possibly influence the dental health standard of the patient [1].

One of the possible mechanisms of such an influence is by the incorporation of trace elements in enamel during tooth development and mineralization. The alternation of trace elements within the structural make up of the enamel hydroxyapatite crystals, influences the physical character of the crystals, hence, rendering them more or less resistant to enamel dissolution during the initiation of the carious process [1,6,23].

Table 2. Variations of trace element concentrations means measured in ppm (\pm Stdv) in sound and carious enamel of primary and permanent teeth ($\eta=5$)**

Trace element	Sound permanent	Cariou s permanent	Significance	Sound primary	Cariou s primary	Significance
Zn	138.7(\pm 2.3)	136.9 (\pm 1.2)	N.S	146.1 (\pm 2.2)	143.4 (\pm 1.4)	N.S
Sr	88.6 (\pm 1.8)	65.7 (\pm 5.4)	$P=3.5 \times 10^{-5}$	82.4 (\pm 2.7)	70.2(\pm 4.9)	$P=9.1 \times 10^{-4}$
Al	72.6 (\pm 2.2)	76.1 (\pm 1.0)	N.S	79.3 (\pm 1.6)	86.1 (\pm 1.3)	$P=1.7 \times 10^{-5}$
Pb	39.2 (\pm 2.2)	41.8 (\pm 3.5)	N.S	42.6 (\pm 1.8)	45.3 (\pm 2.7)	N.S
Cu	36.1 (\pm 1.2)	29.5 (\pm 2.3)	$P=4.1 \times 10^{-3}$	32.6 (\pm 3.7)	22.4 (\pm 4.3)	$P=2.2 \times 10^{-3}$
Fe	30 (\pm 2.6)	21.5 (\pm 3.9)	$P=2.5 \times 10^{-5}$	32.3 (\pm 3.4)	26.9 (\pm 1.8)	$P=8.1 \times 10^{-4}$
Mn	3.3 (\pm 0.2)	4.1 (\pm 0.6)	$P= 1.3 \times 10^{-4}$	4.8 (\pm 1.0)	5.5 (\pm 1.3)	$P=3.3 \times 10^{-3}$
Se	0.45 (\pm 0.1)	0.73 (\pm 0.08)	$P= 3.1 \times 10^{-5}$	0.82 (\pm 0.2)	1.12 (\pm 0.3)	$P=7.8 \times 10^{-3}$

Stdv is the standard deviation. **Units are in ppm (part per million)

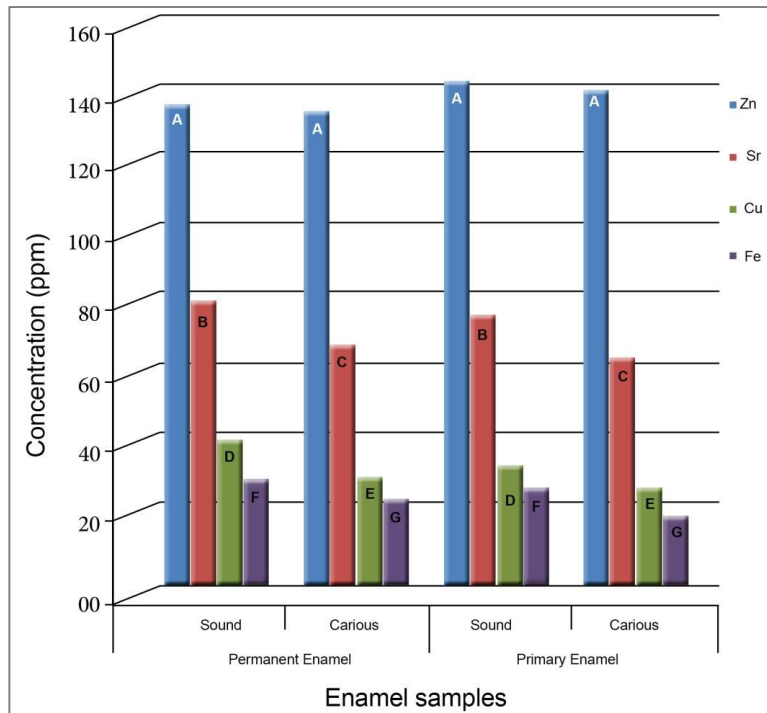


Fig. 4. Histogram representing the concentration (ppm) of Zn, Sr, Cu, and Fe, in sound and carious enamel samples of permanent and primary teeth.
Different alphabets denote significant difference at 95% confidence level ($P < 0.05$)

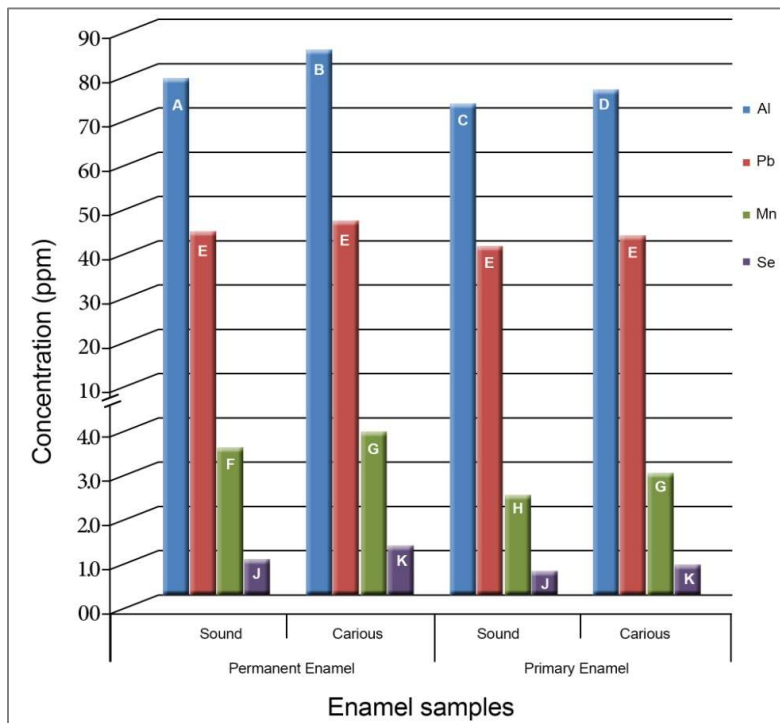


Fig. 5. Concentrations (ppm) of Al, Pb, Mn, and Se in sound and carious enamel of primary and permanent teeth.
Different alphabets denote significant difference at 95% confidence level ($P < 0.05$)

Some trace elements are bound to the mineral structure or organic matrix of enamel at the time of its formation; others are adsorbed on the enamel surface after tooth eruption. Both forms of elements bound to enamel or in the environment, are believed to play a role in the caries process [2,7,9,10,15,16].

The results of the present investigation revealed that the concentration of Al, Mn, and Se in sound teeth were significantly lower than in carious teeth. These results agreed with previously reported findings on the cariogenic effect of Al, Mn, and Se [25,26,27,28]. Curzon and Losee, [26] 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. Saiki et al. [27] reported that the concentration of Mn determined by neutron activation analysis was found higher in carious than in caries-free teeth. Davies and Anderson, [25] confirmed the adverse effect of Selenium, Se, on enamel; it was emphasized that cavity formation in teeth starts as a localized dissolution of the enamel surface by the products of bacterial activity, if this is followed by the incorporation of the kind of trace elements such as, Se into the apatite microcrystals of enamel, it would alter their properties, particularly, solubility, which in turn would influence the crystals' susceptibility to degradation.

Despite that Zn and Pb has, respectively, occupied a high, and an intermediate ranks among the other six trace elements in terms of their presence within the structural make up of enamel of the investigated primary and permanent teeth, the results arrived at for Zn and Pb did not demonstrate any significant concentration differential between sound and carious enamel in both primary and/or permanent teeth. This result trivializes the role played by the two elements in enhancing caries resistance of enamel.

The present results on iron "Fe" indicated association of this element with enamel resistance to caries. Its significantly higher concentration in sound enamel compared to that in carious enamel of both primary and permanent teeth implied that Fe may have inverse association with dental caries. Reintznerova et al. [23] reported that the concentrations of Fe, Cu, and other elements were higher in the surface first layer than in whole enamel and that their concentrations decreased towards the enamel-dentin junction. It was believed that this

concentration gradient may result from interaction between saliva and teeth, therefore, supports the hypothesis that the surface demineralization/remineralization process is effective in surface layers, where trace elements have an increasing or decreasing effect on caries formation. Emilson and Krasse [29] reported caries rate reduction in teeth after introducing an iron salt supplement into the diet. Shashikiran et. al. [6], confirmed that trace elements in human tooth enamel were derived from the environment during mineralization both during and after maturation of teeth.

The obtained results for Copper 'Cu' in the present study demonstrated higher Cu concentrations in sound than in carious enamel of primary and permanent teeth. These results were in agreement with the finding of a previous report by Shashikiran et al. [6].

In the present study, Strontium 'Sr' revealed significantly higher concentrations in sound primary and permanent enamel than in their carious counterparts, highlighting the role played by Sr in enhancing enamel resistance of both primary and permanent teeth against caries. This result is concordant with that reported by Curzon and Losse, [26] who related the high enamel Strontium concentration to low caries incidence. It is believed that Strontium-substituted apatites are harder to remove from enamel than the pure Calcium compound [7], and the Strontium-remineralized enamel is considerably less soluble than enamel remineralized without Strontium. It has been shown that Strontium in solution retards the dissolution rate of hydroxyapatite [30]. Therefore, even if the Strontium-apatite dissolves fast, the subsequent increase in Strontium concentration of the pore solution would still retard the net demineralization rate through a surface phase mechanism, leaving change in the Strontium concentration once remineralization has occurred. The cariostatic role played by Strontium has also been emphasized by the findings of Athanassouli et al. [3] who reported that the incorporation of Strontium in dental enamel renders it more resistant to caries as a result of the hetero-ionic exchange of Calcium in the apatite lattice enhancing the apatite crystals' resistance against demineralization by acids.

4. CONCLUSION

The absolute concentrations of the microminerals Zn, Sr, Al, Pb, Cu, Fe, Mn, and Se were measured by graphite furnace atomic absorption

spectrophotometry in normal and carious enamel from primary and permanent teeth. The concentrations of Sr, Cu, and Fe were significantly higher in sound than in carious enamel, indicating a cariostatic influence of Sr, Cu, and Fe on primary and permanent teeth. On the other hand, 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. Neither Zn nor Pb showed a significant concentration differential between sound and carious enamel which trivialized the role of the two elements in enhancing caries resistance of enamel in primary and permanent teeth.

CONSENT

In compliance with the policy of the clinical research authority at the JUH, signed written informed consents were obtained from all the subjects selected for the study. All subjects were made aware that their extracted teeth were included in the investigation. The experimental protocol was examined and approved by the ethics committee and was, therefore, performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki, last edited in 2013.

ETHICAL APPROVAL

Written consent was obtained from all subjects who were made aware that their extracted teeth are going to be included in the study before the investigation started. The local ethics committee approved the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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