

# ESTIMATION OF THREE MICROELEMENTS IN PRIMARY TEETH BY INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROSCOPY

## ABSTRACT

**AIM:** This study was carried out to estimate the concentrations three microelements in enamel of deciduous normal and carious teeth using atomic emission spectrometry. **METHODS:** A total of 80 deciduous molar teeth indicated for extraction from children between the age group of 7-13 yrs. were taken for this study. The specimens were divided into four groups and the samples were prepared using centrifuge flotation technique. They were analyzed for Manganese, Strontium and Zinc using inductively coupled plasma-atomic emission spectroscopy. **RESULTS:** This study shows that sound enamel contains more of strontium when compared to the carious enamel. Carious enamel contains more of manganese, and zinc when compared to the normal enamel. Elements zinc and strontium occur in high concentrations, whereas elements manganese occur in low concentrations in enamel. The concentrations of elements manganese, strontium and zinc does not vary in relation to sex of individual. **CONCLUSION:** The microelements strontium and zinc are present in high concentration. Further studies are necessary to see their potential in mineralization and anticariogenic process.

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## KEYWORDS

Trace elements. Dental enamel. Atomic emission spectrometry.

## INTRODUCTION

Investigations into the role of micro elements in caries prevention have attracted great interest in the research community in the past. The heterogeneous nature of dental caries cannot be understood without appreciating what influence the process of enamel formation has upon its subsequent chemistry and properties.<sup>1</sup> Trace elements are defined as Inorganic elements present in minute quantities in animal tissues regardless of their abundance in nature. Losee<sup>2</sup> et al. 1974 classified trace elements in dental tissues into three categories: (a) Elements found in dental enamel at a median concentration above 10 µg/g dry wt. [eg] F, Mg, Zn, Sr; (b) Elements found in dental enamel at a median concentration between 1 µg/g and 10 µg/g dry wt [eg] B, Al, Cr, Ba; (c) Elements found in dental enamel at a median concentration between 0.1 µg/g and 1 µg/g [eg] Mn, Cu, Se, Sn.

The composition of enamel is not static; it changes constantly with age, environment, diet, pH of the overlying plaque and caries.<sup>3,4</sup> In the carious tooth the chemical make up of the enamel is altered due to demineralization, vice versa there can be incorporation of certain elements in the carious lesions from natural sources which helps in remineralization. Therefore there can be variations in trace element concentration in normal and carious tooth.<sup>3,4,5,6,7</sup> The hydroxyapatite crystals

making up the enamel may, by changes in trace element composition, become more/less resistant to enamel dissolution during the initiation of caries process. This method has been demonstrated to have synergistic action in enamel dissolution.<sup>8</sup> The incorporation of trace elements may materially effect the subsequent caries initiation in the tooth. So by analysing human enamel, it should be possible to find out the chemical co-relation between trace elements of sound and carious enamel.

A study was therefore conducted to determine and compare Manganese, Strontium and Zinc, in the enamel of sound and carious deciduous teeth of males and females in the age group of 7 to 13 years by Inductively Coupled Plasma-Atomic emission spectrometry.

## MATERIAL AND METHODS

A total of 80 deciduous molar teeth freshly extracted from children between the age group of 7-13 years. were taken for this study. All teeth were indicated for extraction and before extraction parent's consent was taken. These children have same demographic and similar socio-economic status and environmental factors and are not suffering from any systemic illness.

#### CRITERIA FOR SELECTION OF SAMPLE:

Group I: Twenty deciduous sound molar teeth, with out any caries or morphological aberration extracted from male children. These teeth where indicated for extraction due to mobility or over retention; Group II: Twenty deciduous, sound molar teeth. with out any caries or morphological aberration extracted from female children. These teeth were indicated for extraction due to mobility or over retention; Group III: Twenty deciduous, carious molar teeth extracted from male patients, these teeth were indicated for extraction because they were non-restorable or due to over retention; Group IV: Twenty deciduous carious molar teeth extracted from female patients. These teeth were indicated for extraction because they were non-restorable or due to over retention.

Each group is divided into five samples of four teeth each.

#### PREPARATION OF THE SAMPLE:

Freshly extracted molars were thoroughly washed with running water to dislodge the debris and to eliminate the blood materials from the tooth surface. After washing the teeth were allowed to dry naturally. The root portion of the collected tooth were removed with carborandum disc. Each sub group samples were ground into fine powder,

using agate motor and pestle and the enamel dust was separated from dentin using centrifuge flotation technique (Manly and Hodge). The powdered samples are introduced separately into a solution of acetone and bromoform mixture having a specific gravity of 2.7. The samples were centrifuged for 5 minutes at 3000 rpm at room temperature. Enamel having a density of 2.92 settles during centrifugation, while the lighter dentine particles with a density of about 2.40 floats on the surface(Figure 2). The floating dentine particles are discarded with the supernatant liquid. This enamel dust is collected and dried using filter paper and is stored in a polythene bag.<sup>9,10,11</sup>

From each sub group sample, one gram of over dried sample was transferred to a teflon beaker, and 10ml concentrated perchloric acid was added. The sample was then brought very slowly to boiling on a hot plate and heated to dryness. If sample blackening occurred during the fuming stage, nitric acid was added drop wise. The sample was then taken, cooled, redissolved in 10ml double distilled water and 1ml concentrated hydrochloric acid and brought to volume in a 50ml bottles.<sup>11,12,13</sup> Manganese, Strontium and Zinc were analysed using inductively coupled plasma-atomic emission spectroscopy.<sup>14,15</sup>

## RESULTS

This study was carried out to estimate the concentrations of three elements namely, Manganese, Strontium and Zinc in the enamel of deciduous teeth in comparison to normal and carious teeth and to relate the element concentration to the sex of the individual. The data obtained for each element was subjected to statistical analysis for mean value and standard deviation. The differences were

compared using variance ratio analysis ('F' test). Table 1 shows the data obtained for each element for the 20 samples. Sample 1 to V denotes data from male normal teeth, samples VI to X denotes data from female normal teeth, sample XI to XV denotes data from male carious teeth and samples XVI to XX denotes data from female carious teeth.

Table 1. Trace element values of each element.

		Manganese	Strontium	Zinc
Group1	Sample 1	1.23	110.11	304.34
	Sample 11	1.45	128.23	270.41
	Sample 111	1.12	160.2	350.63
	Sample 1V	1.8	114.15	288.1
	Sample V	1.74	119.82	286.39
Group11	Sample V1	1.55	121.18	310.8
	Sample V11	1.67	129.84	287.47
	Sample V111	2.74	144.19	258.38
	Sample 1X	1.43	132.21	298.3
	Sample X	1.35	125.63	288.67
Group 111	Sample X1	1.79	98.73	412.85
	Sample X11	2.33	108.73	408.66
	Sample X111	1.83	87.91	466.31
	Sample X1V	1.64	94.22	397.51
	Sample XV	1.73	93.36	479.82
Group1V	Sample XV1	1.53	96.31	492.38
	Sample XV11	1.54	111.42	483.19
	SampleXV111	1.12	81.37	386.08
	Sample X1X	3.68	88.49	473.44
	Sample XX	1.66	84.89	438.2

Table 2 shows comparison of data using 'F' test. Analysis show carious teeth contains more manganese than the normal teeth which is significant at 5% level whereas strontium and zinc content is more in normal teeth than

in carious teeth which is significant at 1% level. There is no statistical difference in, Manganese, strontium and zinc content in relation to the sex in normal and carious teeth.

## DISCUSSION

Enamel is capable of performing this function only because of its most abundant constituent, hydroxyapatite, which gives it a hardness intermediate between that of iron and carbon steel.<sup>3,6,16</sup> The post eruptive accumulation of some trace element can take

place in sound enamel by adsorption; where as other trace elements accumulate only if the surface or sub surface enamel is hypomineralized due to some developmental disturbance or by demineralization process.<sup>17,18</sup>

Table 2: Comparison of trace elements in Normal and Carious teeth.

ELEMENTS		'F' VALUE	INFERENCE
	Normal Vs Carious teeth	7.03	Significant at 5% level
MANGANESE	Normal teeth (Male Vs Female)	<1	N.S
	Carious Teeth (Male Vs Female)	<1	N.S
	Normal Vs Carious teeth	34.53	Significant at 1% level
STRONTIUM	Normal teeth (Male Vs Female)	<1	N.S
	Carious Teeth (Male Vs Female)	<1	N.S
	Normal Vs Carious teeth	97.44	Significant at 1% level
ZINC	Normal teeth (Male Vs Female)	<1	N.S
	Carious Teeth (Male Vs Female)	1.19	N.S

Trace elements may reduce or potentiate caries.<sup>19,20</sup> The receptivity of apatite lattice to a wide range of elements facilitates their incorporation into the enamel, this reaction occur in the surface layers where the substitution of new elements can influence the solubility of the enamel.<sup>19</sup>

Manganese is a constituent of enamel which appears in higher concentrations in the outer enamel layers than in the inner enamel layers. Manganese is deposited in enamel during calcification and there may be a post eruptive gain from other external sources.<sup>12,18</sup> In the present study it is noted that manganese

content of enamel does not vary with the sex of the individual. This is in confirmity with the results shown by Derise.<sup>4</sup>

Losee<sup>5</sup> stated that the uptake of strontium occurs prior to eruption, probably during tooth formation, and there is no change in the strontium content of the tooth post eruptively. Verbic<sup>21</sup> postulated that the concentration of strontium is about constant in the surface and subsurface enamel. He further stated that strontium salts when administered before and after weaning could reduce the caries formation due to the possibility of substitution of strontium for calcium in the apatite lattice and the adsorption of calcium by the enamel surface. Derise<sup>4</sup> found that the strontium content of teeth decreased with age, and it is significantly greater in younger children than in other age groups. Curzon<sup>23</sup> in a study of trace elements and dental caries demonstrated an association of high strontium content to low carious rates. In the present study the results show that carious tooth has low strontium content than in the sound tooth.

Zinc is deposited in teeth before eruption.<sup>24,26,27</sup> Pre-eruptive deposition is favoured by high concentration of zinc known to be present in extracellular fluid of young individuals and is organically bound to the tooth. The amount of zinc deposited is dependent upon the zinc binding capacity of the proteins in these tissues and the availability of ionic zinc during its deposition.

The inorganic zinc is acquired by the enamel by exchange of calcium in the hydroxyapatite crystals.<sup>4,26</sup> The zinc content in enamel of sound and carious teeth in this study corresponds to the values presented by other studies.<sup>9,10,12</sup> Little<sup>27</sup> on the trace element analysis of carious enamel found zinc content to be significantly higher than in sound tooth, it confirms the findings of the present study.

A comparison of results of the present study with the results of different authors can only be relative. Differences in sample preparation, conditions, and analytical methods contribute to the differences in results. There is no specific analytical methods for estimation of trace elements. In the present study, estimation was done by Inductively coupled plasma atomic emission spectroscopy, which is a standard method of trace element analysis.<sup>14,15</sup>

## CONCLUSION

Based on the recorded data from the present study, following conclusions were made.

1. Sound enamel contains more of strontium when compared to the carious enamel;
2. Carious enamel contains more of manganese, and zinc when compared to the normal enamel;

3. Elements zinc and strontium occur in high concentrations, whereas elements manganese occur in low concentrations in enamel;

4. The concentrations of elements manganese, strontium and zinc does not vary in relation to sex of individual;

Further studies are necessary to see the mineralization and re mineralization potential of strontium.

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