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Assessment of Certain Trace Elements in Human Tooth Enamel in Urban and Rural Regions of Basrah Governorate Utilizing SEM Technology

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ABSTRACT

This current research aims to study the concentrations of certain trace elements in human tooth enamel as a means of detecting environmental pollution in Basra governorate, comparing the results between rural and urban environments and between different age groups. Samples of deciduous and permanent teeth were collected from individuals of both sexes and from multiple regions within the province, and then analyzed using the technique of scanning electron microscopy equipped with energy–dispersive X-ray spectroscopy (SEM-EDX). The findings indicate that the main components of enamel are calcium, phosphorus, and oxygen, and that their proportions vary depending on the age of the individual, housing location, and the type of tooth. Rural samples recorded higher proportions of calcium and phosphorus compared to urban samples, while oxygen and carbon ratios were higher in urban areas. It was also noted that deciduous teeth have different concentrations than permanent teeth, reflecting the different stages of formation and environmental exposure. These results confirm that teeth represent an effective biomarker for assessing the levels of pollution by elements in the environment, and that the variation between regions in Basra governorate reflects the impact of industrial, agricultural, and oil activities on the accumulation of elements in vital tissue.

1. Introduction

In recent decades, environmental pollution caused by heavy elements has become a global issue that attracts the attention of researchers on a large scale due to its serious health effects and its cumulative nature in the tissues of living organisms, which is negatively reflected on human, animal, and plant health [1]. The environmental research in Basrah Governorate has recorded high levels of the heavy metals in the surface soils due to the industrial processes and anthropogenic pollution. A local investigation of 576 surface soil samples of the various sites in Basrah city recently proved the high amount of iron, lead, chromium, zinc, and manganese which indicated certain areas as hotplaces of pollution. This massive environmental pollution can be potentially dangerous to the human body and can help to develop trace elements in the biological tissue even in the dental enamel [2]. Besides the contamination of soil, the pollution of aquatic environments in Basrah city has also been reported

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to have measurable levels of heavy metals. Surface water and sedimentary studies of rivers and local waters indicated that there were lead and mercury in some of the river systems that were near the industrial operations along the Shatt Al-Arab River. This implies that there are unending exposure pathways of the environment that could lead to systematic accrual of the trace elements in the human tissues [3]. The environmental effect is not limited to the presence of toxic heavy metals, but also includes the presence of the essential body mineral elements that are part and parcel in the formation and maintenance of body tissues, especially the dental enamel. These factors are affected by a complicated interaction between environmental, nutritional, and physiological factors, and the combination of these factors determines the chemical composition and structural integrity of enamel. Dental enamel is one of the hardest tissues in a human body and is mostly comprised of calcium phosphate compounds. Other components such as phosphorus, magnesium, sodium, oxygen, and carbon, which are also essential to enamel mineralization and determination of its physicochemical properties, including hardness, stability, and dental caries resistance, are also found there. The balance between these factors and in particular the ratio of calcium-to-phosphorus (Ca/P) is considered to be one of the major indicators to evaluate the quality of enamel mineralization and structural stability [4]. On the other hand, the absorption of elements in the dentin is an important vital indicator, as these elements are absorbed during the stages of tooth formation and calcification and stored for long periods, whether they are basic elements sourced from food, soil, or non-essential elements resulting from environmental pollution, cconsequently, the elemental analysis of the dental enamel by means of new methods, including the scanning electron microscopy (SEM), may provide precise and comprehensive data on the chemical changes in the teeth and the effects of the environmental factors upon the structure of the enamel [5]. The chronic exposure to numerous environmental factors on the other hand can contribute to changes in the ratios of mineral components in body tissues with time that can cause a number of health conditions especially in children and impacts on growth and development. Some of the studies have reported that disproportion in the necessary amounts of mineral components in the teeth can be linked with a higher vulnerability to dental caries and other diseases associated with the nervous system and the key physiological processes [1]. In this regard, human teeth turn out to be a convenient and unique biological evidence to study the alterations in the elemental composition as teeth are hard tissues that are not renewed once formed and have a comparatively stable chemical composition. The characteristic allows one to identify the history of chronic exposure to different environmental factors. In addition, teeth start to develop in the initial life stages and evolve in various times, which enables the examination of the elemental levels to conclude about the time of the environment exposure in child and adulthood [6]. The importance of this research originates from its focus on the environment of Basra governorate, an area that has experienced significant environmental changes over the past decades due to wars, industrial expansion, and oil activities, which have increased the risk of environmental exposure on the elemental composition of dental enamel. Furthermore, the comparison of rural and urban areas in Basra Governorate gives a perfect chance to explore the effect of the local environmental factors on the elemental composition of dental enamel. The lifestyles, the levels of pollution, industrial and agricultural activities and water sources vary greatly among the two settings, and thus this may influence the percentages of mineral elements in the teeth. Thus, analysis of these differences in the environment is necessary to evaluate their effect on human health, and teeth are a good biological marker.The purpose of the study is to measure the levels of the fundamental mineral salts in human dental enamel, which are calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), oxygen (O) and carbon (C) using scanning electron microscopy (SEM). The study also aims to establish the comparison of these concentrations in urban and rural regions of Basra Governorate and in both deciduous and permanent teeth in an attempt to explain the age and environmental differences in dental enamel elemental makeup.

2. Materials and Methods

2.1. Materials used

The materials used in this study included samples of human teeth of various types, such as molars and premolars, collected from individuals of both sexes and from different age groups. The dental samples used in this study were obtained after extraction for purely therapeutic or pathological reasons, and no tooth was extracted specifically for scientific research purposes. verbal consent was taken from patients before using their extracted teeth in medical clinics (Dr1, Dr2, Dr3,...). No personally identifiable data has been registered or used and the information has been kept confidential and used exclusively for scientific research purposes The research also used a nitrogen cutting device with a diamond precision cutting tool to split the samples, an ethanol solution and distilled water to clean them, a low-temperature dryer to dry them, an adhesive to fix the samples on the stand, a conductive material of gold to cover the samples, as well as a scanning electron microscope (SEM) to perform the examination.

2.2. Preparation of Samples for SEM technique examination procedures

In this study, samples of human teeth were collected from individuals of both sexes and different age groups (children, adults under 18 years old, adults from 20 to 40 years old, and more than 40 years old), and from different areas of Basra governorate (Rural and Urban) in the areas of Abu al-khasib and the city center with total number of 119 samples , with three duplicates per sample for the purpose of estimating the percentages of some elements and comparing them according to location and demographic factors. The samples included different types of teeth, such as molars and premolars, and were collected from dental clinic auditors (the Samples were not extracted due to research interests, they were extracted due to therapeutic or pathological reasons), with detailed data recorded on each participant related to age, gender, social and health status, occupation, housing, and the presence of fillings in the mouth. The teeth were installed in the nitrogen-cutting device. Use a precise and sharp cutting tool made of diamonds, such as a fine saw, and start cutting the teeth into small pieces with a size of 2-5 mm. A nitrogen stream was used during shearing to reduce the heat generated. After the cutting was completed, the samples were immersed in an ethanol solution to clean them of impurities. Then clean them with distilled water to remove any residue from the solution. The samples were placed in a dryer at a low temperature of 40-60 °C for complete drying, taking care not to expose them to high temperatures. We used a suitable adhesive to attach the samples to the test stand. The samples were coated with a conductive material, gold, to improve electrical conductivity. Finally, the prepared samples were placed in a SEM and examined according to approved protocols [7].

2.3. Statistical Analysis Software and methods.

The statistical software that was used to analyze the data was standard statistic software. The raw data were initially tabulated and manipulated with the help of the Microsoft Excel program to compute arithmetic means, standard deviations as well as to generate tables and graphs. The sophisticated statistical analysis followed after using the SPSS (Statistical Package for the Social Sciences). Descriptive statistics formed part of the statistical procedures to describe the concentration of the chemical elements in the teeth. The test that determined the normality of the data distribution was the Shapiro-Wilk test. Since the elements had a normal distribution, independent-sample t-tests were utilized in comparing the means of two groups (e.g., males vs. females, urban vs. rural), whereas one-way ANOVA was used in comparing more than two groups (e.g., three or more samples). In cases where the elements were not normally distributed the Mann-Whitney U test was applied to compare two independent groups, and Kruskal-Wallis H test was applied to compare more than two groups. The level of significance of P 0.05 was used to establish statistically significant differences in values, and therefore the impacts of gender, tooth type, age category, and geographic location on the trace and essential elements in the teeth were assessed.

3. Results

3.1. Proportions of items by age, type of tooth and location

It is noted from Table (1) that the proportions of the elements vary depending on the age, type of tooth, and location. The elements calcium (Ca), phosphorus (P), and oxygen (O) represented the largest proportion of the enamel composition of all samples, while the proportions of carbon (C), sodium (Na), and magnesium (Mg) are relatively low, which is to be expected in the mineral structure of the teeth.

Table 1. Quantitative results of the analysis of the constituent elements of tooth enamel in the center and limb samples using the technique of scanning electron microscopy (SEM-EDX)

Sm. Nu	Tooth Type / Age Group / Location	Ca (%)	P (%)	Mg (%)	Na (%)	O (%)	C (%)
A1	Sprocket / Deciduous tooth / Outskirts	35.55	15.46	0.77	0.77	33.33	14.12
A2	Sprocket / More than 40 / Outskirts	32.81	14.73	0.43	1.21	41.43	9.39
A3	Premolars / 20 to 40 / Outskirts	29.91	12.3	1.09	0.57	42.02	14.11
M1	Sprocket / Deciduous tooth / Center	17.36	8.7	1.22	1.08	50.37	21.26
M2	Sprocket / More than 40 / Center	28.93	11.3	0.47	0.62	36.97	21.72
M3	Premolars / 20 to 40 / Center	35.94	12.71	0.38	0.49	37.1	13.37

Analysis of samples collected from Abu al-Khasib revealed that the calcium (Ca) ratios ranged from 29.91% to 35.55%, which is considered elevated compared with certain samples from the central region. In terms of oxygen (O), the maximum percentage observed in the sample from the premolars of adults aged 20 to 40 was 42.02%. In contrast, the percentage was lower in the deciduous molar compared to the permanent one, which recorded 33.33%. Phosphorus (P) exhibited high values ranging from 12.30% to 15.46%, which indicates the integrity of the mineral structure of the teeth. Regarding trace elements such as sodium (Na) and magnesium (Mg), their concentrations were minimal, remaining below 1.5%, although one sample showed a slight increase in sodium at 1.21%. Samples taken from the city center showed clear differences in the concentration of oxygen and carbon compared to the outskirts of the city. The sample M1 (deciduous molar) had the highest oxygen content (50.37%) and the highest carbon content (21.26%), while calcium (17.36%) and phosphorus (8.70%) decreased significantly compared to the rest of the samples. The sample (from 20 to 40 years old) showed the highest calcium value in this group (35.94%), with a percentage P = 12.71%, which indicates a more mature and calcified enamel at this age stage. The sample (over 40 years old) contained moderate proportions of all elements and was close to the values recorded at the outskirts of the city.

The distinctions between the deciduous sample obtained from the center and that from the outskirts are observable. In the central sample, the proportions of oxygen (O) and carbon (C) were elevated at 50.37% and 21.26%, respectively, in contrast to the rural sample, which exhibited a reduction in calcium (Ca) and phosphorus (P) levels at 17.36% and 8.70%. The rural sample, on the other hand, demonstrated higher concentrations of Ca at 35.55% and P at 15.46%.

In the group of (20-40) years, both samples (outskirts) and (center) exhibited a convergence in the concentrations of the main elements Ca, P, and O. Notably, the urban sample was characterized by a slight increase in calcium levels (35.94%) in comparison to the rural sample (29.91%). This balance of values reflects the constancy of the chemical composition of enamel in this category, a stage of life at which the completeness of mineralization and the stability of mineral elements in the teeth are expected.

For the group over (40) years, the sample (outskirts) showed a slight increase in the proportions of phosphorus (P) (14.73%) and calcium (Ca) (32.81%) compared to the sample from the center, which had the ratio of phosphorus (P) (11.30%) and calcium (Ca) (28.93%).

The pie charts presented in Figure (1-3) illustrate the proportions of the primary elements (Ca, P, O, C, Na, Mg) found in dental samples collected from rural regions. It is observed that calcium and phosphorus are the predominant components of the enamel structure, with minor variations in the ratios of oxygen and carbon across the three samples. In contrast, the pie charts depicted in Figure (4-6) below represent the percentages of the key constituent elements of tooth enamel in samples obtained from urban areas (Center). These samples encompass three distinct age groups: Deciduous, individuals aged 20 to 40 years, and those over 40 years of age.

The findings indicate that the elemental composition of the enamel in these samples is predominantly composed of calcium (Ca), phosphorus (P), and oxygen (O), which are the elements that constitute the crystal structure of the hydroxyapatite compound ($Ca_{10}(PO_4)_6(OH)_2$), known for its role in the hardness and chemical durability of teeth.

This particular sample is characterized by elevated ratios of oxygen (O = 50.37%) and carbon (C = 21.26%) in comparison to the other samples, alongside a notable reduction in calcium (Ca = 17.36%) and phosphorus (P = 8.7%).

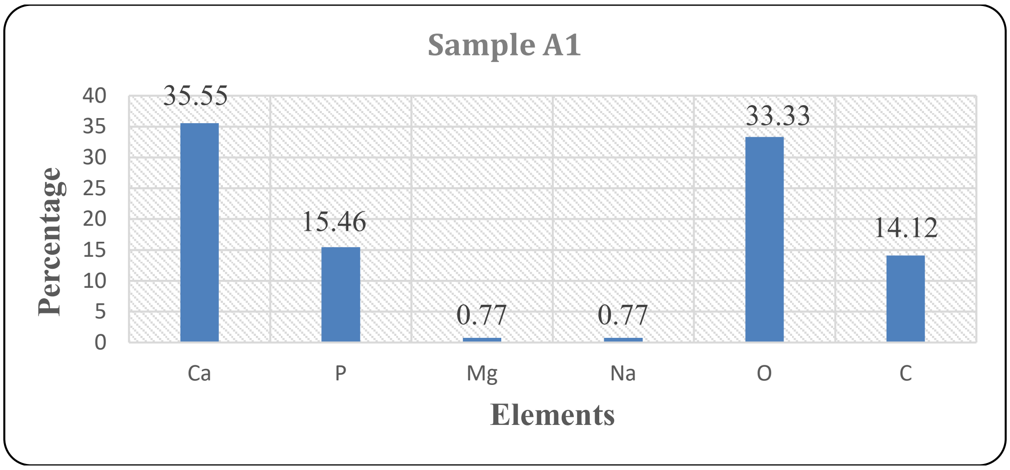

Fig. 1. Relative distribution of the constituent elements of tooth enamel in sample of rural extremities (A1) using the SEM-EDX technique

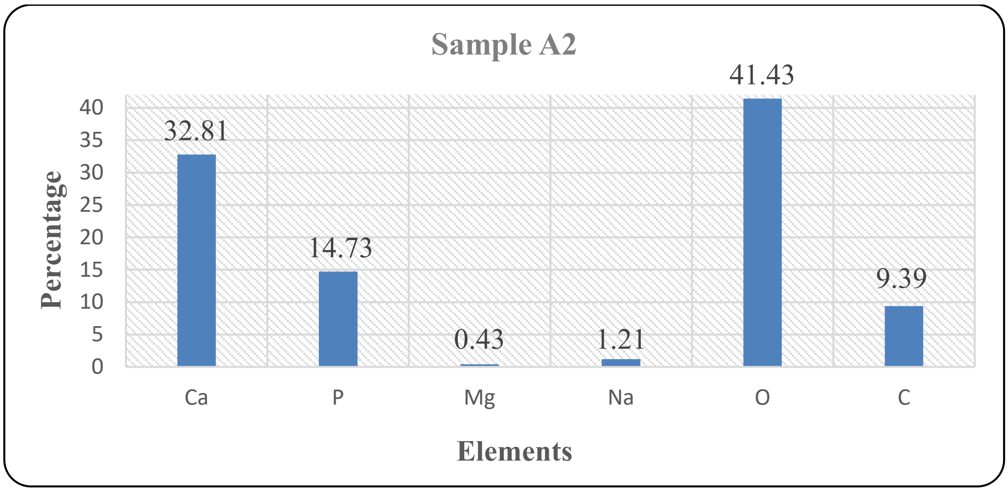

Fig. 2. Relative distribution of the constituent elements of tooth enamel in sample of rural extremities (A2) using the SEM-EDX technique

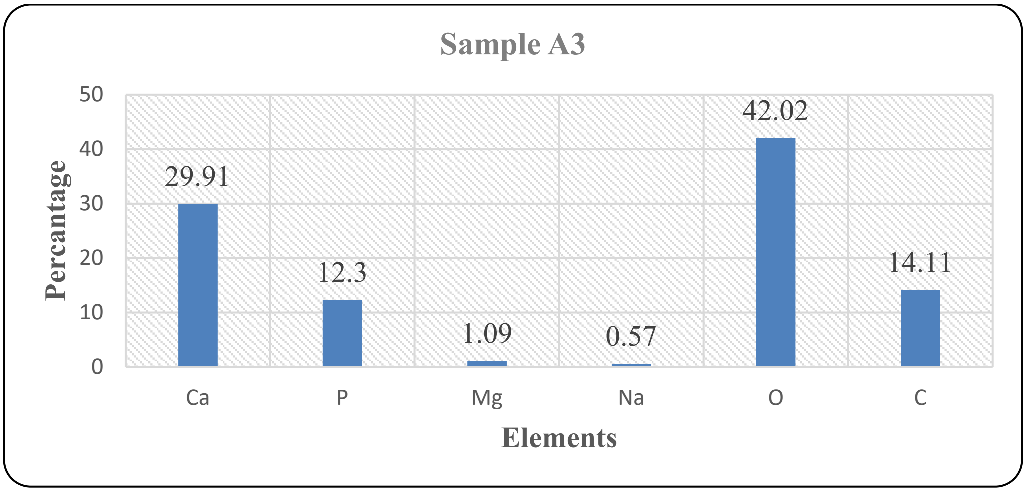

Fig. 3. Relative distribution of the constituent elements of tooth enamel in sample of rural extremities (A3) using the SEM-EDX technique

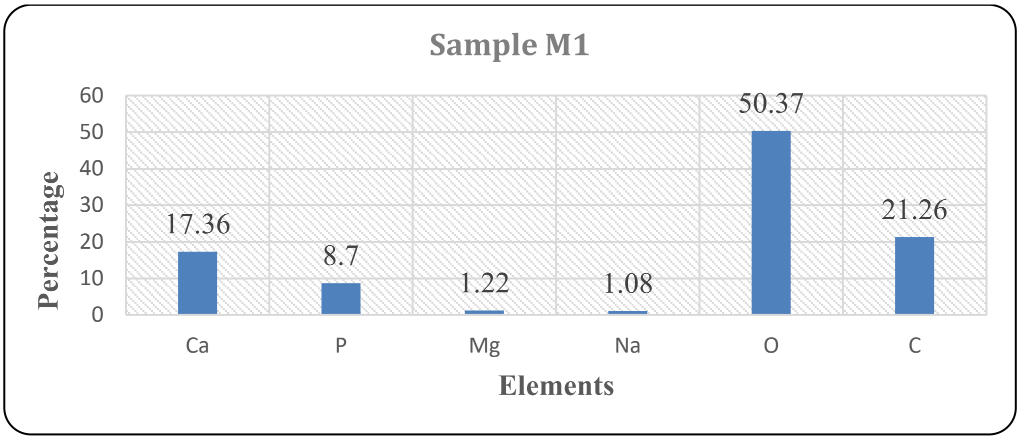

Fig. 4. Relative distribution of the constituent elements of tooth enamel in the center sample (M1) using the SEM-EDX technique

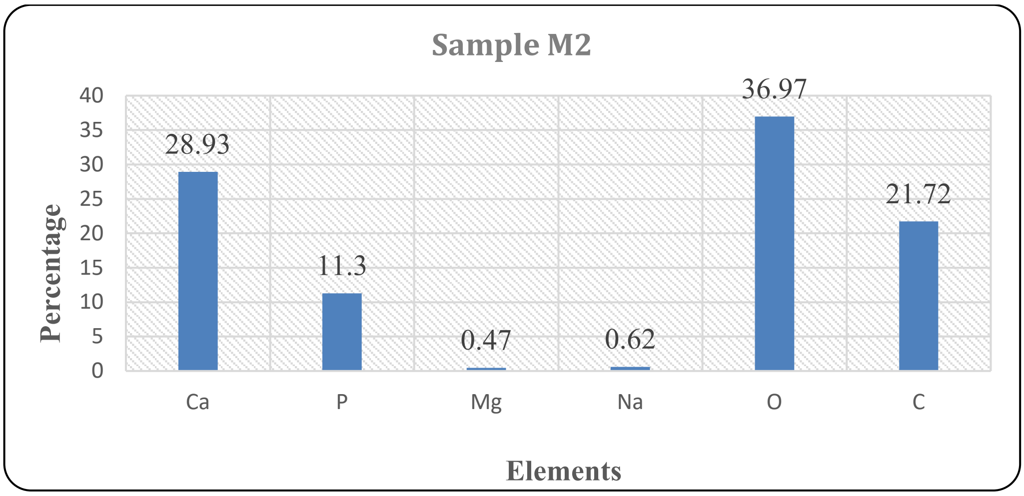

Fig. 5. Relative distribution of the constituent elements of tooth enamel in the center samples (M2) using the SEM-EDX technique

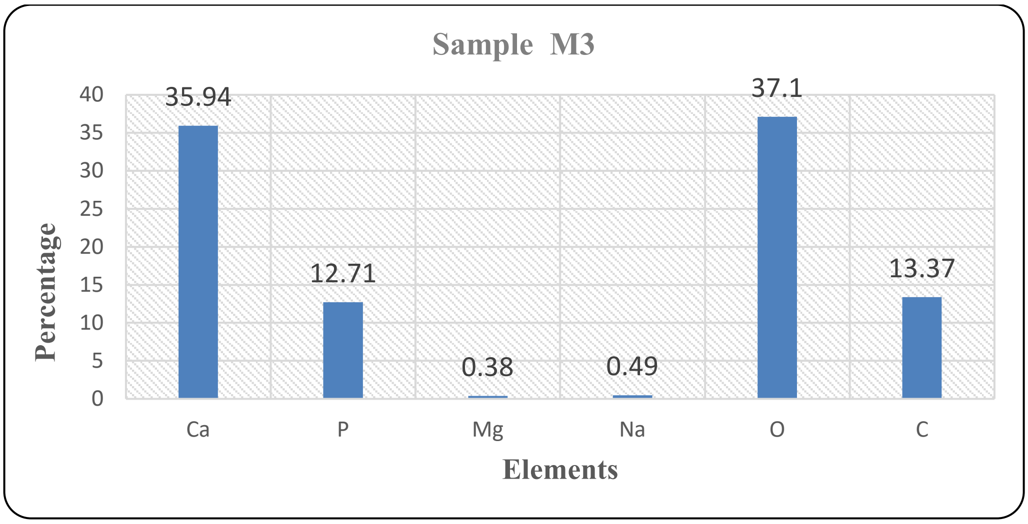

Fig. 6. Relative distribution of the constituent elements of tooth enamel in the center samples (M3) using the SEM-EDX technique

Figure (7) illustrates the graphical representation of each age group alongside their geographical locations (center and outskirts) in a bar chart format, which aids in the comparison among the groups. The figure indicates that the elements carbon (C), oxygen (O), and phosphorus (P) exhibit the highest concentrations across all categories, whereas sodium (Na) and magnesium (Mg) display the lowest ratios. Additionally, a convergence of values among the three age groups is observed.
Figure (3) presents the quantitative findings (W%) of the six elements analyzed: carbon (C), oxygen (O), sodium (Na), magnesium (Mg), phosphorus (P), and calcium (Ca). The samples were collected from two distinct locations representing urban (center) and rural (Abu al-khasib) categories to facilitate a comparison between the two environments. The elements oxygen (O), calcium (Ca), and phosphorus (P) constitute the largest percentage of the W% in the enamel composition across all samples and are the primary constituents of the hydroxyapatite compound ($Ca_{10}(PO_4)_6(OH)_2$), which is the principal component of enamel and dentin. In contrast, the elements carbon (C), sodium (Na), and magnesium (Mg) were found in lower proportions and are classified as secondary or trace elements.
In terms of age group, the findings indicated that the deciduous teeth samples from Abu al-Khasib exhibited the highest calcium (Ca) concentration at 35.55%. In contrast, this concentration diminished to 17.36% in samples collected from the central region. Regarding phosphorus (P), the levels recorded were 15.46% in Abu al-Khasib, whereas the center showed a lower concentration of 8.70%. This data clearly demonstrates a significant increase in both calcium (Ca) and phosphorus (P) levels in the samples from Abu al-Khasib when compared to those from the center.
Conversely, the concentration of oxygen (O) was found to be greater in the deciduous teeth samples from the center, measuring 50.37%, as opposed to the lower concentration of 33.33% found in the samples from Abu al-Khasib.
With respect to trace elements such as sodium (Na) and magnesium (Mg), their concentrations were observed to range between 0.7% and 1.2%, which are considered low and typical in dental bio samples.

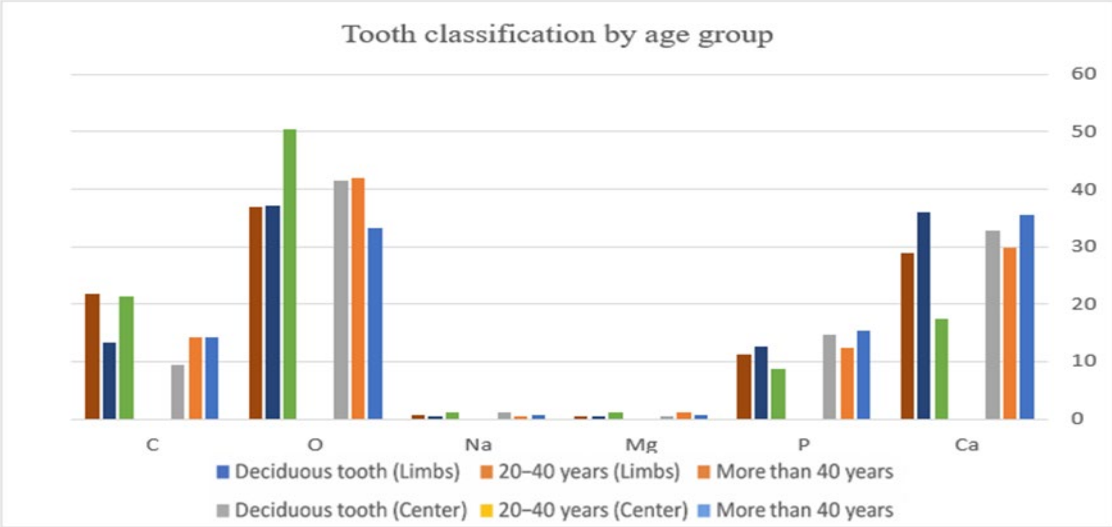

Fig. 7. Age classification by age group

Regarding the age group of 20 to 40 years, this group exhibits a notable consistency in racial composition across the two research locations. The calcium (Ca) content in the central samples was recorded at 35.94%, in contrast to 29.91% found in the outskirts (Abu Khasib). Meanwhile, the phosphorus (P) levels varied slightly, measuring 12.71% in the center and 12.30% in the outskirts.

The element oxygen (O) exhibited the highest concentration in the outskirts at 42.02%, in contrast to 37.10% observed in the center. Regarding carbon (C), the outskirts showed a value of 14.11%, while the center recorded 13.37%. The similar values between the center and the outskirts indicate a level of consistency in the chemical makeup of enamel within this age demographic, which signifies the most stable phase of tooth calcification and maturation.

In the demographic of individuals over 40 years of age, the samples collected from the outskirts exhibited the highest concentrations of calcium (Ca) and phosphorus (P) at 32.81% and 14.73%, respectively, in contrast to the center's samples, which showed 28.93% and 11.3%. Regarding oxygen, a comparable percentage was observed at both locations, with 41.43% in the outskirts and 36.97% in the center. Additionally, a minor increase in the sodium (Na) percentage was noted in the outskirts samples at 1.21%, as opposed to 0.62% in the center.

3.2. Analysis of the Concentration of Essential Elements in the Teeth by Location

Figure (8) shows the weight ratios of the constituent elements of tooth enamel, which were determined using the technique of scanning electron microscopy equipped with an energy–dispersive X-ray analysis spectrometer (SEM-EDX). The main constituent elements of tooth enamel in all samples were calcium (Ca), phosphorus (P), oxygen (O), and carbon (C). In contrast, low proportions of trace elements such as sodium (Na) and magnesium (Mg) were observed. The first four elements are the main components of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which is responsible for the hardness and structural stability of tooth enamel, while sodium (Na) and magnesium (Mg) contribute to the strengthening of the crystalline structure of enamel to a secondary degree.

When comparing the samples collected from the outskirts (Abu al-khasib), it was observed that they exhibit a significant increase in the levels of calcium and phosphorus in comparison to their counterparts from the city center. The average calcium content in the outskirts samples was approximately 32.76%, whereas the center samples showed an average of 27.41%. In terms of phosphorus, the outskirts recorded an average of 14.16%, compared to 10.90% in the center. Conversely, a contrasting trend was noted for oxygen and carbon, with the center samples displaying higher levels of these two elements relative to the peripheral samples. Specifically, oxygen levels in the city center samples reached 50.37% in the deciduous sample, and 37.10% and 36.97% in the adult samples (aged 18-40 and over 40, respectively), while the outskirts samples exhibited lower oxygen levels (33.33%, 41.43%, and 42.02%). Additionally, there was a notable increase in carbon levels in the center samples, particularly in the deciduous samples (21.26%) and the older permanent samples (21.72%), in contrast to the peripheral samples, where carbon ratios varied between 9.39% and 14.12%. In terms of trace elements, all samples exhibited low levels of sodium and magnesium, not surpassing 1.3%, with slight variations. The findings indicated that the calcium to phosphorus (Ca/P) ratio varied from 2.00% in the deciduous sample obtained from the center to 2.83% in the permanent sample from the same location, while it ranged between 2.23% and 2.43% in the outskirts samples. Extremely low percentages were noted for the elements rubidium (Rb), lutetium (Lu), zirconium (Zr), niobium (Nb), tellurium (Te), and aluminum (Al), which were disregarded due to their minimal presence.

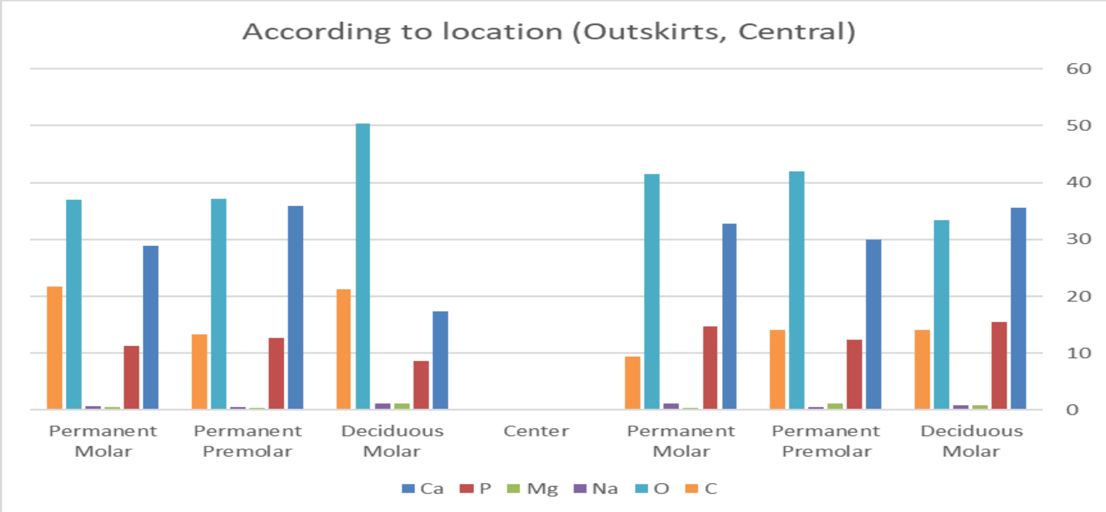

Fig. 8. Comparison of the percentages of the elements composition in the tooth enamel based on the type of tooth (deciduous molar, permanent molar, permanent premolar), and the place of living (outskirts and central).

3.3. Distribution of Elements statistically

The purpose of this section is to describe and discuss the statistical findings of the measurement of the concentration of a series of chemical elements in dental samples of people representing two age groups (children and adults) and two geographic regions in the Basra Governorate which are urban areas (city center) and rural areas (Abu Al-Khaseeb). To accomplish the statistical analysis, the descriptive statistics of each element, in the form of the mean, standard deviation, median, minimum and maximum values and the range, were calculated. To determine the normality of the distribution of each of the variables, the Shapiro-Wilk test was performed. As a result of small sample size restrictions and possible heterogeneity in certain variable distribution, Kruskal-Wallis test was to be used to compare multiple groups, and most variables were found not to be normally distributed.

3.3.1. Descriptive Statistics

Statistical analysis using SPSS provided the mean values of the chemical elements (C, Ca, P, Mg, Na, O) where the arithmetic mean was the mean of the values of the measured elements in the samples of the dental materials. Table (2) shows the descriptive statistics of the levels of these chemical components in the six dental samples that were studied namely carbon (C), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and oxygen (O). The outcomes have shown that the mean concentration of oxygen (O) was the highest of the elements and was 40.20 percent, then the concentration of calcium (Ca) was 30.08 percent and the concentration of phosphorus (P) was 12.53 percent. The mean amount of carbon (C) was 15.66 percent with a standard deviation of 4.84 percent which shows that there was a significant difference between the various samples as seen by the variation between the lowest and highest amounts of 9.39 percent and 21.72 percent respectively. Other trace elements had not so high concentrations as the major elements with magnesium (Mg) and sodium (Na) averages of 0.73% and 0.79% respectively (SD = 0.36 and 0.29).

The medians of the elements were nearly equal to their means value indicating that there was no strong skewness distribution. Particularly, the median of oxygen was 39.27% and calcium 31.36 which are very close to their respective means. Conversely, carbon had the highest dispersion of the elements with a standard deviation of 4.85 which was the highest comparison against all other elements and this implies that there are wide variations of the carbon levels in each of the six samples.

Table 2. Descriptive Statistics of Dental Sample Chemical Elements Concentrations.

Elements	The Number of the Samples (N)	(Mean)	Standard Division (SD)	(Median)	Minimum Value	Maximum Value
Carbon (C)	6	15.6617	4.8459	14.115	9.39	21.72
Calcium (Ca)	6	30.0833	6.8536	31.36	17.36	35.94
Phosphors (P)	6	12.533	2.4368	12.505	8.7	15.46
Magnesium (Mg)	6	0.7267	0.3609	0.62	0.38	1.22
Sodium (Na)	6	0.79	0.2926	0.695	0.49	1.21
Oxygen(O)	6	40.2033	5.9244	39.265	33.33	50.37

3.3.2. Elementary Concentration Normality Test

Table (3) shows the outcomes of the normality tests on the concentrations of the chemical elements of the dental samples, on both Kolmogorov–Smirnov and the Shapiro-Wilk test. The tests were performed to determine how much the data of each element follow a normal distribution, and this is a condition of using parametric tests based on the assumption of normal distribution. The findings suggest that the P-values (Sig.) on each of the elements used in the Shapiro-Wilk test are greater than the level of significance of 0.05 indicating that the data on the elemental concentration are not significantly different. The test statistics varied between 0.842 and 0.962, which was near the desired value of 1.0, which further proved the regularity of the data and its approximate normality. In the same manner the results of the Kolmogorov-Smirnov test revealed a similar trend, all the p-values were above 0.05 which revealed that there is no significant deviation of the measured elements out of normality. The findings suggest that the concentration of the chemical elements (carbon, calcium, phosphorus, magnesium, sodium and oxygen) conform to a pattern, which is very close to a normal distribution in the sample under study.

Table 3. Normality Test of Dental Sample Chemical Elements Concentrations.

Elements	Kolmogorov–Smirnov (Statistic)	df	Sig.	Shapiro–Wilk (Statistic)	df	Sig.
Carbon (C)	0.291	6	0.121	0.876	6	0.253
Calcium (Ca)	0.267	6	0.2	0.842	6	0.135
Phosphors (P)	0.15	6	0.2	0.962	6	0.838
Magnesium (Mg)	0.262	6	0.2	0.868	6	0.218
(Na)Sodium	0.219	6	0.2	0.896	6	0.35
Oxygen(O)	0.213	6	0.2	0.927	6	0.559

3.3.3. Elemental Concentration Comparison between Ages

In Table (4), the findings of hypothesis testing used the Independent-Samples Krustal-Wallis Test to evaluate the statistically significant differences in the concentrations of the major elements investigated such as the calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), oxygen (O), and carbon (C) among various age groups. The age groups were deciduous and permanent teeth consisting of people who were below the age of 18 years, between 18 and 40 years, and above 40 years. The findings revealed that all the p-values of these elements were greater than the value of the significance level of 0.05 with a span of 0.180 to 1.000 of sodium (Na) to phosphorus (P). In particular, the p-values were 0.651, 0.368, 0.867, and 0.565 of calcium (Ca), magnesium (Mg), oxygen (O), and carbon (C). The null hypothesis of all tests was accepted because all the p-values were more than 0.05, and the assumption is that elemental concentrations distribution is equal in all age groups. These results imply that the levels of these major elements were significantly similar in teeth across the age groups and tooth types under study, which implies that age and tooth-type (deciduous/permanent) did not have a significant effect on the variation in the basic mineral composition of teeth in the sampled population. In general, the inferential statistical analysis showed that the differences between age groups were not statistically significant in relation to any of the elements investigated ($p > 0.05$).

Table 4. Summary of Hypothesis Testing to Compare Distribution of Chemical Elements between the age groups using the Independent Kruskal-Wallis test.

Elements	Sig.	Decision	Interpretation By Age
Carbon (C)	0.65	Failure to reject the Null hypothesis	Equally ,there was no significant difference in the amount of carbon (C) in the age groups.
Calcium (Ca)	1	Failure to reject the Null hypothesis	The calcium (Ca) distribution was also comparable in all age groups, and therefore it appeared that there was no evident impact of the age on the calcium (Ca) concentration.
Phosphors (P)	0.37	Failure to reject the Null hypothesis	The concentration of phosphorus (P) was the same in all the age groups indicating that age did not affect its concentration .
Magnesium (Mg)	0.18	Failure to reject the Null hypothesis	The age groups did not show any statistically significant difference and magnesium (Mg) did not vary much in different ages.
Sodium (Na)	0.87	Failure to reject the Null hypothesis	There were some slight differences but they were not found to be significantly different.
Oxygen (O)	0.57	Failure to reject the Null hypothesis	The oxygen (O) distribution was heterogeneous in all the age groups, with no noticeable impact of the age in its content.

3.3.4. Comparison of Major Elemental Concentrations by Location.

Table (5) demonstrates that the significance values (Sig.) of all of them were above 0.05 and therefore, no statistically significant difference was observed in the concentration of chemical elements across the various parts of the study. This implies that, the proportions of the key chemical elements are equal and uniform at the sampled locations and that there is no noticeable impact of the location on the concentrations of the elements. The P-value of calcium (Ca) was 0.513, which showed that the concentration of calcium in the two areas of study (city center and Abu Al-Khaseeb) was similar, which is a reflection of the similarity in the environment that affected the accumulation of calcium. The P-value of phosphorus (P) was 0.127 and it was not below the significance level, which means that there was no significant result in the amount of phosphorus present in the different locations. Magnesium (Mg) p-value of 0.827 showed no significant difference in its distribution, possibly because it is relatively stable, and it is related to similar geological factors or similar organic factors in all sites. In the same way, sodium (Na) had a p-value of 0.513 that revealed similar concentrations in the various locations. Oxygen (O) p-value=0.827 and similar distribution across the sites was observed and this is not surprising because Oxygen is a key element of most organic and inorganic compounds that indicates the overall chemical stability of the samples. The P-value of carbon (C) was 0.275, which is above 0.05, showing that there is no statistically significant difference between the distribution of carbon at the locations. All in all, the obtained results indicate a similar distribution of all of the elements under study in the various locations, and the effect of geographic location on the levels of the elements under study was weak or statistically nonsignificant. The comparison of the urban and rural samples revealed that no statistically significant differences were observed between the elemental concentrations at the locations ($p > 0.05$).

Table 5. The Kruskal-Wallis Test of the Distribution of Chemical Elements by Location.

No.	Elements	Test Name	Sig.	Decision	Scientific Interpretation
1	Carbon (C)	Independent-Samples Kruskal–Wallis Test	0.51	Failure to reject the Null hypothesis	there were no significant differences in the levels of carbon (C) in the locations studied.
2	Calcium (Ca)	Independent-Samples Kruskal–Wallis Test	0.13	Failure to reject the Null hypothesis	The distribution of calcium (Ca) was not found to show any significant differences at the various locations.
3	Phosphors (P)	Independent-Samples Kruskal–Wallis Test	0.83	Failure to reject the Null hypothesis	The phosphorus (P) also did not differ significantly across sites despite slight and insignificant differences.
4	Magnesium (Mg)	Independent-Samples Kruskal–Wallis Test	0.51	Failure to reject the Null hypothesis	Magnesium (Mg) was uniformly distributed throughout all the locations meaning that there was consistency in the level of magnesium.
5	Sodium (Na)	Independent-Samples Kruskal–Wallis Test	0.83	Failure to reject the Null hypothesis	There were no statistically significant differences between sites with sodium (Na
6	Oxygen (O)	Independent-Samples Kruskal–Wallis Test	0.28	Failure to reject the Null hypothesis	Oxygen (O) was found to be equally distributed in all the places, as it is comparatively stable.

4. Discussion

The findings from the analysis of the elemental ratios in tooth enamel across various age groups, types of teeth, and geographical regions reveal a series of chemical patterns that can be interpreted through the lens of biological and environmental processes influencing tooth formation and development. These findings hold significant importance as they illustrate the intricate interplay between biological factors (including age and type of tooth) and environmental influences (such as dietary habits, water quality, and geographical context), along with the impact of behavioral and health-related factors that may directly or indirectly modify the mineral composition of enamel [6]. General data suggest that tooth enamel exhibits minor variations in the proportions of certain fundamental mineral elements, including calcium (Ca), phosphorus (P), magnesium (Mg), and sodium (Na), as well as the organic structural components such as carbon (C) and oxygen (O). Calcium and phosphorus constitute the foundation of the hydroxyapatite compound ($Ca10(PO4)6(OH)2$), which imparts hardness and significant chemical and mechanical resistance to the enamel. The elevated levels of these two elements in the majority of samples indicate effective mineralization and enhanced crystalline stability of the enamel. This observation aligns with findings reported by [9], which indicate that calcium and phosphorus play a crucial role in the processes of demineralization and remineralization of teeth. The ideal concentration and levels of calcium and phosphorus within the body serve to fortify the teeth's defense mechanisms and mitigate the risk of caries. The disparities observed between samples collected from urban and rural regions distinctly illustrate the influence of environmental factors. Teeth sourced from rural locations generally exhibit slightly higher levels of calcium and magnesium, potentially attributable to the mineral-rich nature of groundwater or the consumption of traditional diets abundant in minerals. The elevated pH (acidic nature) of river and well water may enhance the solubility of calcium in teeth, thereby expediting the demineralization of enamel [10]. Conversely, certain samples from urban regions demonstrate a comparatively lower content of calcium and phosphorus. This phenomenon can be elucidated by the urban population's exposure to factors such as the consumption of processed foods or carbonated drinks, which contribute to a reduction in mineralization levels due to their high acidity [11,12]. It is well-established that individuals in rural areas frequently depend on traditional foods that are rich in calcium and phosphorus, including dairy products and fish, which facilitate the calcification of enamel during the formative stages of milk teeth [13]. M1 sample (a tooth of the city center that was also a deciduous one) was also characterized by a significant decrease in the calcium and phosphorus levels to 17.36% and 8.70, respectively, as compared to the other samples where higher values were registered. The decrease was accompanied by a significant increase in the oxygen (50.37%) and carbon (21.26) percentages. This result may be explained by the fact that the level of mineralization is lower and the proportion of organic components of teeth is higher in the case of the latter and therefore more vulnerable to the processes of demineralization and corrosion of hydroxyapatite crystals, resulting in reduced Ca and P levels and the accumulation of non-mineral components [14]. Moreover, the effect of environmental pollutants that lead to accelerated wearing of the tooth enamel according to the urban origin of the sample can also be realized [15]. In addition, the surface sensitive character of the SEM-EDX method makes the results vulnerable to surface changes induced by dental caries or acid erosion and this way gives explanations to the observed values without necessarily being a sign of an error in analysis. Conversely, trace elements like sodium (Na) and magnesium (Mg) play a secondary yet important role in enamel composition. They help improve the uniformity of the hydroxyapatite crystal lattice and strengthen the enamel's resistance to mechanical stress. Small variations in the ratios of these elements may be linked to local water sources, as highlighted by [16], who indicated that water rich in sodium and magnesium can influence mineral levels in dental tissues. Zamzam water, known for its high concentrations of sodium, calcium, and magnesium, has shown significant effects in remineralizing tooth enamel that has undergone industrial demineralization. The application of Zamzam water results in increased micro-hardness similar to that achieved with sodium fluoride treatment. Additionally, it is important to note that differences in elemental proportions across age groups are due to physiological changes associated with aging. Age is a key factor affecting the structure of dental hard tissues and their chemical makeup, as concluded by [17]. As people age, the structure of enamel and the ionic processes within it change. The hydroxyapatite content decreases with age, and the lattice constant related to carbonate content in carbonate apatite systematically decreases over time, while the lattice constant c remains relatively stable [18].

As age increases, calcium and phosphorus levels rise, while organic components decrease, resulting in a harder and more durable enamel. The similar ratios of elements found in most samples from the two study regions suggest environmental uniformity within the Basra governorate. This indicates that water and nutritional sources do not differ significantly between urban and rural areas, and the consistent element ratios in tooth enamel reflect stable environmental conditions in the same geographical area. Based on these findings, teeth could serve as an inexpensive and simple biological method for detecting chronic pollution in local communities.

5. Conclusion

The current study gives a detailed account of the elemental composition of human dental enamel in the samples determined in urban (city center) and rural (Abu Al-Khaseeb) parts of Basra Governorate as well as between various age groups and types of teeth. The results show that the main components of enamel are calcium (Ca), phosphorus (P) and oxygen (O), which constitute the crystal lattice of hydroxyapatite that guarantees the hardness and structural integrity of the organs, whereas carbon (C), sodium (Na) and magnesium (Mg) are the secondary or trace elements. Statistical analysis showed that the elemental concentrations were mostly similar among the ages, types of teeth (deciduous and permanent) and geographical regions. The Kruskal-Wallis tests and the normality tests showed that there was no significant difference between the groups of study in the distribution of calcium, phosphorus, magnesium, sodium, oxygen, or carbon. These findings indicate that there is no significant difference in the fundamental mineral structure of enamel in the given population due to the age, the kind of tooth, and rural or urban localities. The difference in elemental ratios between the urban and the rural samples might be due to small environmental and dietary components, as in that urban teeth had a bit more calcium and phosphorus materials, possibly because of the mineral-rich water and conventional diet. On the other hand, the carbon and oxygen content of the urban samples were higher and may be explained by the lifestyle, dietary, and exposure to processed foods. These relatively small differences do not affect the overall elemental composition, which serves as evidence of the enamel and its ability to maintain its chemical integrity in different environmental conditions. This paper has shown that human teeth represent a good biological marker in tracking the presence of essential minerals as well as the possible environmental factors. The results highlight the possible application of the dental enamel as a non-invasive biomarker to determine the chronic exposure to environmental factors and to add to the improved explanation of human health and nutrition in the Basrah Governorate.

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Conflict of interest

The authors report there are no competing interests to declare

Consent for publication

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تقدير بعض العناصر النزرة في مينا الأسنان البشرية في المناطق الحضرية والريفية لمحافظة البصرة باستخدام تقنية المجهر الإلكتروني الماسح

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الملخص

يهدف هذا البحث الحالي إلى دراسة تركيزات بعض العناصر النزرة في مينا الأسنان البشرية كوسيلة للكشف عن التلوث البيئي في محافظة البصرة ، ومقارنة النتائج بين البيئات الريفية والحضرية وبين الفئات العمرية المختلفة. تم جمع عينات من الأسنان اللبنية والدائمة من أفراد من كلا الجنسين ومن مناطق متعددة داخل المحافظة ، ثم تم تحليلها باستخدام تقنية المسح المجهري الإلكتروني المجهز بالتحليل الطيفي للأشعة السينية المشتتة للطاقة. تشير النتائج إلى أن المكونات الرئيسية للمينا هي الكالسيوم والفوسفور والأكسجين ، وأن نسبها تختلف حسب عمر الفرد وموقع السكن ونوع السن. سجلت العينات الريفية نسبا أعلى من الكالسيوم والفوسفور مقارنة بالعينات الحضرية ، بينما كانت نسب الأكسجين والكربون أعلى في المناطق الحضرية. ولوحظ أيضا أن الأسنان اللبنية لها تركيزات مختلفة عن الأسنان الدائمة ، مما يعكس المراحل المختلفة للتكوين والتعرض البيئي. تؤكد هذه النتائج أن الأسنان تمثل مؤشرا حيويا فعالا لتقييم مستويات التلوث بالعناصر في البيئة ، وأن التباين بين المناطق في محافظة البصرة يعكس تأثير الأنشطة الصناعية والزراعية والنفطية على تراكم العناصر في الأنسجة الحيوية.

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