გზით წინასწარ აპრობირებული ნახევრად-სტრუქტურირებული კითხვარის გამოყენებით. პოტენციურად შეუსაბამო რეცეპტების გამოვლენისათვის გამოყენებული იყო START/STOPP-ის კრიტერიუმები. პოლიფარმაცია გამოვლინდა ხანდაზმული ადამიანების 36,7%-ში. გამოკვლეული პოპულაციის მიერ გამოყენებული წამლების საშუალო რაოდენობამ შეადგინა 6,19±2,43. ყველაზე გავრცელებული დაავადება გამოკვლეულ პოპულაციის ხანდაზმულ მოსახლეობაში იყო ართრიტი (34,9%), შემდგომ - ჰიპერტონია (13%). არასწორი დანიშნულება და შეცდომები პოლიპრაგმაზიის სერიოზულ პრობლემას ქმნის. წინამდებარე კვლევაში წარმოდგენილია მხოლოდ საწყისი მონაცემები შეუსაბამო დანიშნულებების და პოლიპრაგმაზიის შესახებ. ამ პრობლემის რადიკალური გადაწყვეტისათვის, ხანდაზმული ადამიანების ფარმაკოთერაპიის ოპტიმიზებისა და, შესაბამისად, ჯანმრთელობის დაცვის გაუმჯობესებისათვის აუცილებელია მრავალმხრივი მიდგომა საზოგადოებივი ჯანდაცვის, კანონმდებლების და პოლიტიკოსების მონაწილეობით.

X-RAY SPECTRAL ANALYSIS OF DENTAL HARD TISSUE TRACE ELEMENTS (ELECTRON-MICROSCOPIC EXAMINATION)

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Of the 92 chemical elements occurred naturally, 81 are found in the human body. 12 of them are called structural elements, since 99% of them are the human body composition elements. These elements are: Carbon (C), Oxygen (O), Hydrogen (H), Nitrogen (N), Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg), Sulfur (S), Phosphorus (P), Fluorine (F) and Chlorine (Cl). Trace elements are present in very small amounts in a human body – approximately 1 mg/kg of body weight, compared to structural elements. Trace elements can be divided into four main groups: vital (essential), conditionally essential, conditionally toxic and toxic [7,20,29].

Such a division of trace elements is conditional. Studying their structure and "nature" strengthened the veracity of the dictum by Paracelsus, a founder of Iatrochemistry - "There are no toxic substances, but there are toxic doses" [11].

The role, trace elements play in maintaining oral health has not been fully investigated and still remains the subject of research and discussions. Some trace elements promote caries development, while others, conversely, prevent the formation of this process and accelerate the restoration of tooth hard tissues [7,13,18,24].

Whether one or another trace element plays a specific role in maintaining oral health, they are indispensable and serve an important function in vital physiological processes in a human body [10,17].

Mineralization of the dental hard tissues takes place during tooth formation. Enamel has a stable and unchanging composition, while dentin, maturation degree of which is determined by the ratio between mineral/organic components and water, undergoes a change. This change, occurring within adjacent to the pulpal area - predentin, is the result of exposure to pulp microbial infection, trauma, or iatrogenic factors. Final result is a mineralized transformation of the dentin tissue in this region. That is probably the reason why dentin (at different stages of development and transformation), unlike enamel, has several names: primary, secondary, substitutive, reparative, and so on [1].

Mineralization degree and structural changes in dental tissues are determined precisely by trace element ions. They contribute to the formation of hypo- or hyper-mineralized dentine layers. For example, Lead (Pb), when "embedding" into hydroxyapatite, disrupts the mineralization process, giving a rise to enamel demineralization [6,23].

Enamel demineralization, as well as remineralization, is considered as a phenomenal feature, since enamel is the most highly mineralized of the four tissues of inorganic structures in the human body (bone, enamel, dentin, and cementum), and its lattice is represented by apatite crystals: Ca (PO)(OH) Hydroxyapatite, Ca (PO)CO Carbon Apatite, Ca (PO) Cl Chlorapatite and Ca Sr (PO) Strontium-Apatite. The most common among them is hydroxyapatite [1,14,29].

Dissolution (demineralization) of pH-induced apatite crystals in saliva is often substituted by the reverse process – remineralization. This process is based on sufficient concentrations of Ca and P and pH neutralization in saliva. Remineralization process is accelerated by the presence of fluoride ions.

Fluoride ions easily replace the hydroxyl group (due to equal ionic radius, charge and hydration degree) to form fluorapatite. The latter is stronger and more acid-resistant than hydroxyapatite. As a result, an impenetrable barrier to various substances or ions is created, consequently reducing the enamel conductivity.

The penetration of ions into the hydroxyapatite crystals is a complex process and proceeds in several stages. The first phase involves ion exchange process between saliva and hydroxyapatite crystal hydrated membrane. This stage ends quickly, in just a few minutes. The ions move so fast that they often cannot even penetrate the crystal and are localized only in its surface layer (e.g. Sodium and Fluoride ions).

The second phase involves ion exchange between hydrated membrane of the crystals and the surface layer.

During this stage, the ions, "torn off" from the crystal surface, are substituted by other ions. This process leads to decrease or neutralization of the surface charge. At this stage of enamel mineralization, Ca, F, Sr, Na, P ions attach to apatite surface layer, which is a relatively long process. At the final stage (of unspecified duration), ions settle in the depths of hydroxyapatite crystals, increasing the strength of the latter.

The enamel permeability depends on such factors as the volume of microspaces, ions or molecules within and between the crystals, as well as their ability to attach to enamel components.

For instance: Fluoride ions (0.13 nm), penetrating the enamel and binding to the elements of damaged layer, hardly reach the deeper layers. Calcium (0.18 nm), on the contrary, is easily adsorbed, passes via the crystal lattice and diffuses into the deeper layers of the enamel. Iodine ions (0.136 nm), like calcium, easily penetrate into the enamel but are not able to bind to hydroxyapatite crystals. Iodine easily crossing the dentin barrier, penetrates into the pulp tissue, from where via the blood stream it is carried to the thyroid and adrenal glands [29,30].

In view of the above, it will be evident that trace elements which are able to "build" dental hard tissue structures, apatite crystals, directly affect the degree of tissue strength, thereby changing its stability and resistance to injuring factors.

Consequently, penetration of trace elements into dental structures through saliva, food, water and other routes contributes to the formation of carious diseases, or conversely, its cessation and/or regression.

One of the most important elements in the process of caries development, having anti-caries effect, is - Fluorine (F). It is a chemically strong, active, nonmetallic and the most potent oxidant. The estimated average fluoride concentration in the human body is 7 ppm. 99% of it is presented in the form of fluorapatite. Fluorine-containing compounds are absorbed in the body via food and water. Fluoride is concentrated in rice, beef, eggs, milk, onions and spinach. Tea and fish are particularly rich in fluoride.

Actually, Fluorine does not occur free in the body (as well as in nature), it is always in combination with other minerals. Fluorine forms insoluble salts (Fluorides) with Calcium, Magnesium or Iron. In total, an adult human body contains about 2.6g of fluoride. Fluoride is distributed in all tissues, although 99% of the total body fluoride is found in bones and dental enamel.

A pronounced anti-caries effect of Fluoride created the need for its addition to toothpastes, gels or mouthwashes. It has been found that Fluoride concentration of 0.03 ppm in saliva is sufficient to initiate the remineralization process, while maintaining this concentration continuously the maximum anti-caries effect can be achieved. It should also be noted that local Fluorides have a short-term effect. They increase the Fluoride concentration in saliva for 2 hours. After this period, the Fluoride concentration in saliva, necessary to affect the enamel, decreases and its replenishing with new portion is required. Stability of fluoride concentration also depends on the individual features of saliva secretion and swallowing. Using Sodium Fluoride tablets showed different results. These medicines (Zymafluor, Fluoretten, Bimbovit fluoro) have age-specific dosing and route of administration. It has been shown that regular intake of NaF tablets ensures Fluoride optimal concentration in saliva from 6 to 12 hours [2,3,8,21,28].

Element Molybdenum (Mo) is also characterized by anti-caries effect. According to the study results in children of regions where drinking water contained a certain concentration of Molybdenum, caries prevalence rate was significantly lower compared to the control group. The same was supported by the study carried out in New Zealand, where due to the high content of Molybdenum in soil and, consequently, in vegetables and grains grown there, the caries incidence rate appeared to be low [17,26].

Literature reviews suggested the data from the studies of the anti-caries effect of Vanadium (V) on experimental animals. It was found that hamsters being administered Vanadium orally or parenterally, while on a cariogenic diet were less likely to develop caries.

The anti-caries effect of Vanadium has also been found in the studies conducted on rats [17], however, the contradicting information was suggested in the study by Hadjimarkos D. M. et all, this year, where monkeys drinking Vanadium-containing water showed increased incidence of dental caries [12].

According to the researchers, high Strontium (Sr) content in the human body significantly reduces caries intensity.

Barmes DE, Adkins BL, and Schamschula RG found out that actually, Strontium ratio is higher in the healthy enamel, as well as in young people compared to the elderly [5].

A correlation between the presence of Lithium (Li) and low caries incidence rate has been also revealed [17].

There are numerous studies on the cariogenic effect of trace elements available in medical literature.

For example, having studied Selenium (Se) effect on dental tissues, its degrading effect on dentin structures has been revealed. A high caries incidence rate has also been reported in patients receiving Selenium [25].

The study on rats showed that the Cadmium (Cd) exposure during the tooth formation process caused a significant increase of caries incidence rate after tooth eruption. However, similar injuries were not observed in rats receiving cadmium-containing preparations after tooth eruption and formation [4].

Lead (Pb) is of a special note. It is known that dental tissue is capable of accumulating Lead from the environment. Lead ions negatively affect Ca and P metabolism causing hypercalcemia and hyperphosphatemia. In children with elevated Lead levels, the incidence of developing hypoplasia is high [9]. A study by Needleman HL et al. reported a correlation between hyposalivation caused by high Lead content in the body and increasing numbers of carious teeth in young children [19].

Following the scientific literature review on micronutrients promoting or preventing caries development, we were interested in epidemiological studies reporting correlation between oral health and the presence of various micronutrients [15-17,22,27].

Analyzing the studies allowed us to conclude that there is very scarce information in the literature about the layered, zonal distribution of "vital" trace elements in healthy (intact) teeth dentin and enamel. However, to study the distribution of caries-static elements (Ca, F, P) on the enamel surface as well as in para-pulpal dentin is of great importance as well.

Based on the foregoing, the purpose of present study was to identify trace elements in human teeth (enamel, dentin and cementum), as well as to determine their localization and concentration.

To reach this objective, it was aimed to study the distribution of elements in human extracted teeth on 6 sites/locations: enamel surface layer, enamel thickness, enamel-dentin border, parapulpal dentin, root dentin, and cementum. The concentration of trace elements at 3-3 points of each above-mentioned location/site (conventionally referred to as "spectrum") has been studied. In total, 18 such locations/sites have been studied (Figs. 1, 2, 3).

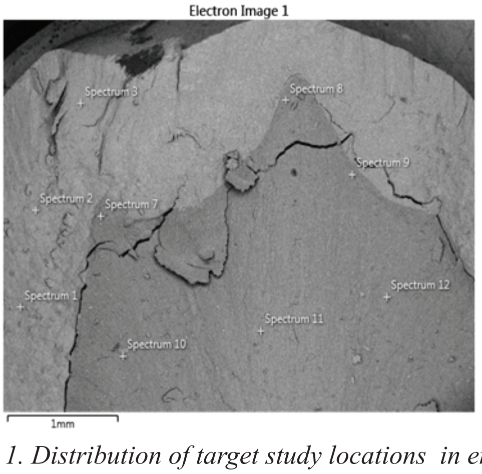

Fig. 1. Distribution of target study locations in enamel and coronal dentin areas

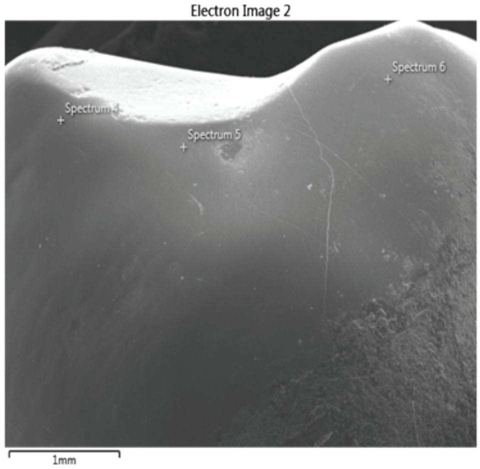

Fig. 2. Target study sites/locations marked in enamel surface layer

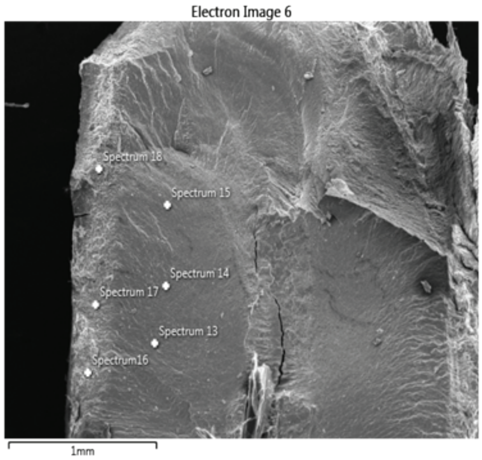

Fig. 3. Distribution of target study locations in tooth root dentin and cementum areas

Material and methods. 6 extracted, single-root, undamaged human teeth have been selected for the study. The indication for tooth extraction was its avulsion (traumatic injury), orthodontist decision, or periodontal disease. The patients' age ranged within 16 to 60 years. Extracted teeth were stored in accordance with the State Standard National Protocol for Clinical Management, "Infection Prevention when Handling of Human Extracted Teeth, of Surrounded Tissue Biopsy and Operative Specimen" 2020.21.02, # 01-282 /o.

The elemental analysis was performed by JSM-6510LV scanning electron microscope (JEOL, Ltd. Japan) equipped with an EDX Genesis energy dispersive X-ray elemental analysis system (X-Max). Electron imaging was performed on both secondary (SEI) and reflected (BEC) electrons with an acceleration voltage of 20 kV.

It should be emphasized that the atoms of any material (including teeth) are characterized by specific spectrum of radiation, wavelengths and intensity. This principle, underlying the methods of X-ray elemental analysis, makes it possible to determine elemental composition of substances.

Table 1. Distribution of trace elements in enamel and coronal dentin (WT, ratio of each element in total weight, %)

Spectrum Label	Spec-trum 1	Spec-trum 2	Spec-trum 3	Spec-trum 7	Spec-trum 8	Spec-trum 9	Spec-trum 10	Spec-trum 11	Spec-trum 12
C	2.19	4.98	4.64	9.23	9.81	2.05	8.40	7.07	9.32
N	0.90	0.00	0.34	7.55	6.06	1.41	7.22	2.73	3.74
O	30.98	47.42	37.14	36.98	38.32	6.93	37.99	43.19	47.64
F	0.06	0.04	0.00	0.02	0.00	0.00	0.23	0.02	0.04
Na	0.41	0.60	0.56	0.55	0.65	0.03	0.54	0.76	0.82
Mg	0.20	0.19	0.17	0.61	0.80	0.08	0.63	1.03	0.91
P	17.37	15.03	17.84	13.54	14.46	9.92	13.30	15.24	12.91
Cl	0.29	0.19	0.29	0.05	0.13	0.10	0.06	0.05	0.02
Ca	47.61	31.53	39.02	31.47	29.77	79.49	31.64	29.91	24.60
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 2. Distribution of trace elements in enamel surface layer (WT, ratio of each element in total weight, %)

Spectrum Label	Spectrum 4	Spectrum 5	Spectrum 6
C	6.74	7.41	8.80
N	3.46	2.21	3.24
O	53.54	54.20	57.52
F	0.30	0.17	0.27
Na	0.44	0.57	0.49
Mg	0.07	0.04	0.07
P	12.20	12.41	10.64
Cl	0.40	0.39	0.38
Ca	22.84	22.60	18.59
Total	100.00	100.00	100.00

Table 3. Distribution of trace elements in root dentine and cementum (WT, ratio of each element in total weight, %)

Spectrum Label	Spectrum 13	Spectrum 14	Spectrum 15	Spectrum16	Spectrum 17	Spectrum 18
C	13.22	12.54	12.72	15.54	13.69	7.48
N	7.23	7.48	8.83	10.00	8.25	5.03
O	48.69	48.74	45.46	47.61	42.30	21.72
Na	0.96	0.98	0.82	0.92	0.91	0.63
Mg	0.57	0.62	0.77	0.71	0.67	0.75
P	10.09	10.25	10.77	8.57	11.20	18.35
Ca	19.24	19.39	20.62	16.63	22.98	46.04
Total	100.00	100.00	100.00	100.00	100.00	100.00

The distance from the electrons outlet tubing to the experimental tooth specimen surface was 15 mm.

In order to reduce the surface charge, specimens were coated with a 10 nm thick Platinum film using vacuum coater (JEOL, Ltd. Japan).

The analyzer software allows converting the result of data analysis into Word document, reflecting the X-ray spectrum of the areas marked on the study material (both in mass and atomic percentages) as well as tables, elements identified at target sites and their concentrations.

The quantitative data given in the tables were statistically processed, indicating the mean square deviation (MSD).

The study showed that among the essential trace elements, the highest concentration of Calcium (Ca) was found in dental hard tissues. Most of it was distributed in the enamel, especially in its thickness (Table 1, 2). As for dentin, more Calcium was found in its coronal part compared to the root (Tables 1, 3).

Analysis of Phosphorus (P) content was performed separately.

Quantitatively, this element is the second most abundant among the trace elements presented in the dental hard tissues, after Calcium. The highest concentration of Phosphorus was described in enamel thickness (Tables 1, 2). The amount of this element in coronal dentin; root dentin was not significantly different (Tables 1, 3). The quantitative values for Oxygen (O) and Carbon (C) content given in the tables are noteworthy. We refrain from proving the accuracy of these data, since it is well known that any object's surfaces in contact with the environment (including experimental - teeth) are "impregnated" with these two elements.

Nitrogen (N) content appeared to be much higher in root dentin and cementum compared to coronal dentin (Table 1, 3), while quantitative values of Sodium (Na), Magnesium (Mg) and Chlorine (Cl) contents were significantly lower in all studied areas of the experimental specimens (Tables 1, 2, 3).

Defining content and localization of the element Fluoride (F) deserves special attention. It turned out that the lowest concentration of Fluoride (F) was observed in all eighteen locations of all study samples. Its concentration ranged from 0.04 to 0.4, while at some locations it was undetectable (Table 1,2,3).

This fact is of a special interest since the presence of Fluorine both on the surface and inner enamel is considered as a precondition for caries prevention.

Consequently, if the Fluoride concentration on the human tooth surface is below the minimum, or absent, this will indicate a lack or ignorance of caries prevention measures. On the other hand, to observe how stable the trace element – Fluorine's retention is in the tooth structure at active application of anti-caries drugs and agents, is of great importance.

Future research will be devoted to solving this issue and all its attendant problems.

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SUMMARY

X-RAY SPECTRAL ANALYSIS OF DENTAL HARD TISSUE TRACE ELEMENTS (ELECTRON-MICROSCOPIC EXAMINATION)

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The role of trace elements (microelements) in maintaining oral health has not been fully investigated and still remains the subject of research and discussion. Some trace elements contribute to the development of caries, while others, on the contrary, prevent formation of this process and accelerate the restoration of dental hard tissues. Penetration of trace elements into human dental structures via saliva, food, water and other routes contributes to the formation of carious diseases, or, conversely, its cessation and/or regression.

Analyzing the studies allowed us to conclude that there is very scarce information available in the literature about the layered, zonal distribution of "vital" trace elements in healthy (intact) teeth dentin and enamel. However, to study the distribution of caries-static elements (Ca, F, P) on the enamel surface as well as in para-pulpal dentin is of great importance as well. It was aimed to identify trace elements in human teeth structures (enamel, dentin and cementum), as well as to determine their localization and concentration.

To reach this objective, X-ray spectral analysis on 6 intact, extracted teeth has been performed by Scanning Electron Microscopy (SEM). Identification of trace elements was performed on the 6 sites/locations of these teeth: enamel surface layer, enamel thickness, enamel-dentin border, parapulpal dentin, root dentin, and cementum. As a result, it has been found that the distribution of essential trace elements in dental hard tissues is uneven, while such an important element in maintaining healthy teeth as Fluorine has been found in only minimal concentrations in hard tissues.

Keywords: trace elements (microelements), dental hard tissues, trace elements in human teeth structures.

РЕЗЮМЕ

РЕНТГЕНОСПЕКТРАЛЬНЫЙ АНАЛИЗ МИКРОЭЛЕМЕНТОВ В ТВЁРДЫХ ТКАНЯХ ЗУБА (ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ)

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Роль микроэлементов в поддержании здоровья полости рта до конца не изучена и часто является предметом исследования и дискуссии. Одни микроэлементы способствуют развитию кариеса, другие, наоборот, препятствуют образованию этого процесса и ускоряют восстановление твёрдых тканей зуба. Наличие микроэлементов в структурах зуба через слюну, пищу, воду и другие формы способствует формированию кариозного заболевания или его прекращению и/или регрессу.

Поскольку в литературе мало сведений о послойном, зональном распределении «жизненно важных» микроэлементов в эмали и дентине здоровых (интактных) зубов, а изучение распределения кариесстатических элементов (Ca, F, P) на поверхности эмали и в парапульпарном дентине весьма значимо, целью исследования явилось идентифицировать микроэлементы в зубах человека, определить их локализацию и концентрацию.

Для достижения поставленной цели проведен рентгеноспектральный анализ удаленных зубов человека под электронным сканирующим микроскопом. В результате установлено, что распределение эссенциальных микроэлементов в твердых тканях зуба неравномерно. А такой значимый для поддержания здоровья зубов элемент, как фтор, содержится в твердых тканях лишь в минимальных количествах.

რეზიუმე

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; სტომატოლოგიის კლინიკა და სასწავლო-კვლევითი ცენტრი "უნიდენტი"; საქართველოს ტექნიკური უნივერსიტეტის სტრატეგიული განვითარების ცენტრი, თბილისი, საქართველო

მიკროელემენტების როლი პირის ღრუს ჯანმრთელობის შენარჩუნებაში ბოლომდე შესწავლილი არ არის და ხშირად კვლევის და დისკუსიის საგანს წარმოადგენს. ზოგიერთი მიკროელემენტი ხელს უწყობს კარიესის განვითარებას, ზოგიც პირიქით, ხელს უშლის ამ პროცესის ჩამოყალიბებას და აჩქარებს კბილის მაგარი ქსოვილების აღდგენას. კბილის სტრუქტურებში მიკროელემენტების ნერწყვით, საკვებით, წყლით და სხვა სახით მოხვედრა კარიესული დაავადების ჩამოყალიბებას, ან პირიქით, მის შეჩერებას და/ან უკუგანვითარებას უწყობს ხელს. გამომდინარე იქედან, რომ ლიტერატურაში საკმაოდ მწირია ინფორმაცია ჯანმრთელი (ინტაქტური) კბილების მინანქარსა და დენტინში „სასიცოცხლო" მიკროელემენტების შრეობრივი, ზონური გავრცელების შესახებ, ამავდროულად კი ჩვენთვის მეტად საყურადღებოა კარიესსტატიკური ელემენტების (Ca, F, P) მინანქრის ზედაპირზე და პარაპულპურ დენტინში განაწილების თავისებურებათა ცოდნა, მიზნად დავისახეთ ადამიანის კბილებში (მინანქარში, დენტინსა და დუღაბში) მიკროელემენტების იდენტიფიკაცია და მათი ლოკალიზაციისა და კონცენტრაციის დადგენა. ამ მიზნის მისაღწევად ელექტრონული მასკანირებელი მიკროსკოპით ჩატარდა ადამიანის 6 ინტაქტური, ექსტრაგირებული კბილის რენტგენოსპექტრული ანალიზი. მიკროელემენტების იდენტიფიკაცია კბილთა 6 სხვადასხვა უბანზე - მინანქრის ზედაპირულ შრეში, მინანქრის სისქეში, მინანქარ-დენტინის საზღვართან, პარაპულპურ დენტინში, ფესვის დენტინსა და დუღაბში განხორციელდა. კვლევის შედეგად დადგინდა, რომ ესენციური მიკროელემენტების გადანაწილება კბილის მაგარ ქსოვილებში არათანაბარია. ხოლო ისეთი მნიშვნელოვანი ელემენტი კბილების ჯანმრთელობის შენარჩუნებაში, როგორიც ფტორია, მაგარ ქსოვილებში მხოლოდ მინიმალური რაოდენობით აღმოჩნდა.

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